SCIENTIFIC OPINION

Scientific Opinion on nitrofurans and their metabolites in food[1]

EFSA Panel on Contaminants in the Food Chain (CONTAM)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Nitrofurans are antimicrobial agents not authorised for use in food-producing animals in the European Union. Nitrofurans are rapidly metabolised, occurring in animal tissues as protein-bound metabolites. The European Commission requested EFSA to provide a scientific opinion on the risks to human health related to the presence of nitrofurans in food and whether a reference point for action (RPA) of 1.0 µg/kg for the marker metabolites is adequate to protect public health. Data on occurrence of nitrofuran marker metabolites in food were extracted from the national residue monitoring plan results and from the Rapid Alert System for Food and Feed (RASFF). The CONTAM Panel concluded that these data were too limited to carry out a reliable human dietary exposure assessment. Instead, human dietary exposure was calculated for a scenario in which a single nitrofuran marker metabolite is present at 1.0 µg/kg in foods of animal origin, excluding milk and dairy products. The mean chronic dietary exposure for this worst-case scenario would range from 3.3 to 8.0 and 1.9 to 4.3 ng/kg b.w. per day for toddlers and adults, respectively. Nitrofurans and their marker metabolites, generally, are genotoxic and carcinogenic and, also, have non-neoplastic effects in animals. Margins of exposure (MOEs) were calculated at 2.0×10^5 or greater for carcinogenicity and at 2.5×10^3 or greater for non-neoplastic effects. The CONTAM Panel concluded that it is unlikely that exposure to food contaminated with nitrofuran marker metabolites at or below 1.0 µg/kg is a health concern. A scenario in which foods are considered to be contaminated with semicarbazide, from use of carrageenan as a food additive, at 1 µg/kg was used to assess whether it is appropriate to apply the RPA to foods of non-animal origin; MOEs of greater than 10^4 calculated for non-neoplastic effects do not indicate a health concern.

KEY WORDS

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nitrofurans, nitrofuran marker metabolites, semicarbazide, food, reference point for action, non-allowed pharmacologically active substance, risk assessment

SUMMARY

Nitrofurans are synthetic broad spectrum antimicrobial agents. The nitrofurans considered in this opinion are furazolidone, furaltadone, nitrofurantoin, nitrofurazone and nifursol. Nitrofurans are not authorised for use in food-producing animals in the European Union (EU), but furazolidone, nitrofurantoin and nitrofurazone may be used in human medicine.

Nitrofurans share a nitrofuran ring which is coupled to a side-chain via an azomethine bond. The side-chains differ for the various drugs, being 3-amino-2-oxazolidinone (AOZ) for furazolidone, 3-amino-5-methylmorpholino-2-oxazolidinone (AMOZ) for furaltadone, 1-aminohydantoin (AHD) for nitrofurantoin, semicarbazide (SEM) for nitrofurazone, and 3,5-dinitrosalicylic acid hydrazide (DNSH) for nifursol. Nitrofurans have short half-lives in animals and therefore they do not occur generally as residues in foods of animal origin. Reactive metabolites are formed that are able to bind covalently to tissue macromolecules, such as proteins and DNA. When animal tissues are consumed as food, the side-chains may be released from the metabolites, namely AOZ, AMOZ, AHD, SEM and DNSH.

The EFSA Scientific Opinion, titled '*Guidance on methodological principles and scientific methods to be taken into account when establishing Reference Points for Action (RPAs) for non-allowed pharmacologically active substances present in food of animal origin*', identified an approach for establishing RPAs for various categories of non-allowed pharmacologically active substances. However, the opinion also identified certain categories of non-allowed pharmacologically active substances that are considered to be outside the scope of the procedure, including substances that are high potency carcinogens, such as nitrofurans. As nitrofurans are excluded from that opinion, and taking into account that the presence of SEM in food may be from sources other than use of nitrofurazone, the European Commission (EC) requested the European Food Safety Authority (EFSA) for a scientific opinion on the risks to human health related to the presence of nitrofurans and their metabolites in food. The opinion should include (a) an evaluation of the toxicity of nitrofurans and their metabolites for humans, considering all relevant toxicological endpoints and identification of the toxicological relevance of nitrofurans and their metabolites present in food, and (b) an exposure assessment of the EU population to nitrofurans and their metabolites from food, including the consumption patterns of specific (vulnerable) groups of the population. In addition, the opinion should assess the appropriateness of using marker metabolites of nitrofurans for the reference point for action for food of animal origin. The opinion should evaluate whether a reference point for action of 1 µg/kg for nitrofuran metabolites, as defined in legislation, in food of animal origin is adequate to protect public health, and it should assess the appropriateness of applying the reference point for action, considered adequate to protect public health, to other commodities than food of animal origin.

Because the nitrofuran parent compounds can only be detected in animal tissues and products for a short period after treatment of the animals, monitoring of nitrofuran residues in livestock based on the identification of the parent compounds is not appropriate. Metabolites binding covalently to proteins and persisting for several weeks in edible tissues, from which the side-chains AOZ, AMOZ, AHD, SEM and DNSH may be released, serve as excellent marker metabolites for the illicit use of nitrofurans in food-producing animals. Generally, both screening and confirmatory methods for the nitrofuran marker metabolites in foods of animal origin use acid hydrolysis and nitrobenzaldehyde derivatisation of the released marker metabolites. Screening for the resulting nitrophenyl derivatives is generally undertaken by enzyme-linked immunosorbent assays (ELISA) or biosensor methods, providing sufficient analytical sensitivity to meet the current minimum required performance limit (MRPL) of 1 µg/kg. Confirmatory methods are based on liquid chromatography–tandem mass spectrometry (LC-MS/MS) and also adequately meet the MRPL of 1 µg/kg.

The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) concluded that, since other nitrofuran metabolites that could persist at higher concentrations have not been identified, the marker metabolites AOZ, AMOZ, AHD, SEM and DNSH are appropriate as the RPA for foods of animal origin.

Data on occurrence of nitrofuran metabolites (AOZ, AMOZ, AHD and SEM) in food, reported by Member States from the National Residue Monitoring Plans, have been extracted for the period 2002 to 2013; there were 214 non-compliant targeted samples reported for nitrofurans over that 12 year period. The categories in which nitrofurans were reported in decreasing level of incidence were poultry, bovines, sheep/goats, pigs, farmed game, honey, rabbit, aquaculture, horses and wild game. Data were extracted also from the Rapid Alert System for Food and Feed (RASFF) database for the years 2002 to 2014; there were 808 notification events reported for nitrofuran metabolites (AOZ, AMOZ, AHD and SEM), of which 416 were for crustaceans and products thereof and 150 were for poultry meat and poultry meat products.

The CONTAM Panel concluded that data extracted from the EC database and the RASFF database were too limited to carry out a reliable human dietary exposure assessment. Instead, the CONTAM Panel calculated the hypothetical human dietary exposure for a scenario in which foods of animal origin, excluding milk and dairy products, are considered to contain one nitrofuran marker metabolite at a concentration level equal to the RPA of 1 µg/kg. This scenario, representing a worst-case situation for the occurrence of nitrofuran marker metabolites due to illicit nitrofuran use, is a highly unlikely situation. The mean chronic dietary exposure across dietary surveys for this scenario would range from 1.9 to 4.3 ng/kg b.w. per day for adults and would be the highest for toddlers, at 3.3 to 8.0 ng/kg b.w. per day.

Besides arising from nitrofurazone use, SEM may occur in food from other sources, including use of the food additive carrageenan. The CONTAM Panel considered scenarios covering the different sources. In one exposure scenario, foods of animal origin (including only those milk and dairy products for which carrageenan is authorised as an additive) and foods of non-animal origin for which carrageenan is authorised as an additive, were included. These foods are considered to be contaminated with SEM at a concentration level equal to the RPA of 1 µg/kg; this scenario covers all potential dietary exposure. The mean chronic dietary exposure to SEM across dietary surveys for this scenario would range from 6.4 to 16 ng/kg b.w. per day for adults and would be the highest for toddlers, at 17 to 55 ng/kg b.w. per day.

Reduction of the nitro group seems to be the most important metabolic pathway for nitrofurans, potentially leading to reactive intermediates that are capable of binding to proteins and to DNA. Nitroreduction and subsequent redox-cycling results in the generation of reactive species (including oxygen species) that might be responsible for some of the adverse effects.

Based on studies with radiolabelled nitrofurans, high levels (mg/kg range) of metabolites are present in tissues shortly after the last treatment. A proportion of the metabolites cannot be extracted from the tissues with organic solvents and are assumed to be protein-bound. Levels of these residues decrease gradually but are still detectable after 45 days in muscle, kidney and liver of treated pigs and probably for much longer. The decrease of residues in liver and kidney is faster than in muscle tissue.

Feeding of rats with protein-bound residues of radiolabelled furazolidone showed that some of the radiolabel was excreted in urine and so must have been absorbed in the gastrointestinal tract. The radiolabel was also detected in tissues of rats and was partly non-extractable. AOZ could be released by acid treatment from these non-extractable residues in rat tissues. Free AOZ was detected in blood of rats fed with meat containing only protein-bound residues of furazolidone, showing that AOZ can also be released from these residues, probably due to acid hydrolysis in the stomach.

Acute toxicity studies in laboratory animals showed that for furazolidone, nitrofurantoin and nitrofurazone the lung is an important target for toxicity, leading to decreased respiratory function and death. Signs of neurotoxicity such as hyperirritability, tremors and convulsions were also found.

In repeated dose toxicity studies, AOZ caused hepatotoxicity, decreased body weight gain and anaemia at the lowest tested dose of 0.9 mg/kg b.w. per day in rats and at 1 mg/kg b.w. per day in dogs. Nitrofurantoin caused toxic effects in liver, kidney and testes, and caused necrosis of the ovarian follicles, decreased weight gain and neurotoxicity, with a no observed adverse effect level (NOAEL) of about 120 mg/kg b.w. per day in rats and mice. Nitrofurazone caused similar effects as nitrofurantoin, with the exception of necrosis of the ovarian follicles, and the NOAEL for effects on the testes in rats was 13.5 mg/kg b.w per day. SEM caused severe deformation of limbs and osteochondral lesions at the lowest tested dose of 23 mg/kg b.w. per day in rats. Nifursol caused slight changes in red blood cell parameters and a NOAEL of about 14 mg/kg b.w. per day was identified.

In studies on spermatogenesis, furazolidone, furaltadone, nitrofurantoin and nitrofurazone caused toxic effects on the testes in rats and mice but no NOAEL could be identified. Effects were observed at the lowest dose tested of 10 mg/kg b.w. per day for nitrofurantoin.

In studies on embryotoxicity and teratogenicity, furazolidone in mice was embryotoxic at the lowest dose tested of 200 mg/kg b.w. per day and caused decreased body weight and viability of pups after birth, but no malformations were found. Nitrofurantoin was embryotoxic in mice and rats and caused decreased body weight and viability of pups after birth. A NOAEL of 10 mg/kg b.w. per day was identified for embryotoxicity in rats. Malformations were not found in offspring of rats and rabbits, with a NOAEL of 30 mg/kg b.w. per day for teratogenicity. Nitrofurazone was not teratogenic in mice and rabbits at doses that were not maternotoxic. For fetotoxicity/maternotoxicity an overall NOAEL of 14 mg/kg b.w. per day was identified. For SEM, in a study looking at the incidence of cleft palate and resorptions only, an effect was found when rats were treated orally with SEM at 25 mg/kg b.w. per day or higher, but not when treated at 10 mg/kg b.w. per day.

In multigeneration studies, nitrofurazone showed reproductive toxicity in mice for two generations at doses of 14 to 102 mg/kg b.w. per day. Nifursol did not have any effects on reproduction in rats treated for three generations at doses of 54 mg/kg b.w. per day or lower.

In studies on neurotoxicity, nitrofurantoin caused peripheral nerve damage in rats treated orally at the lowest dose tested of 20 mg/kg b.w. per day. SEM caused neurobehavioural effects in juvenile rats when treated orally at the lowest dose tested of 40 mg/kg b.w. per day for 10 days.

In genotoxicity studies, furazolidone and its marker metabolite AOZ were found to be genotoxic *in vitro* and possibly also *in vivo*. Since AOZ can be released from bound residues of furazolidone metabolites, these bound residues should be considered as genotoxic. Furaltadone was found to be a bacterial and mammalian cell mutagen *in vitro*. The marker metabolite AMOZ is not genotoxic *in vitro*. *In vitro*, nitrofurantoin induces mutations, chromosomal aberrations and DNA damage and, *in vivo*, nitrofurantoin has been shown to induce DNA damage in multiple organs, micronuclei formation in mice and gene mutations in a transgenic mouse mutation assay. For AHD, the only *in vivo* mutagenicity study which is available shows a negative result. Nitrofurazone and its marker metabolite SEM are genotoxic *in vitro*. *In vivo* tests gave negative results with nitrofurazone, whereas no conclusion can be drawn on the *in vivo* genotoxicity of SEM. Nifursol is genotoxic *in vitro*, whereas *in vivo* it induced neither chromosomal aberrations nor mutations.

In chronic toxicity and carcinogenicity studies, furazolidone induced malignant mammary tumours in rats, bronchial adenocarcinomas in male and female mice and neural astrocytomas in male rats. The CONTAM Panel concluded that furazolidone is carcinogenic in mice and rats. No information on the carcinogenicity of AOZ, the marker metabolite of furazolidone, was identified, but it is presumed that AOZ may play a role in tumour formation. Furaltadone induced malignant mammary tumours in female rats. The CONTAM Panel concluded that furaltadone is carcinogenic in rats. There is no

information on the chronic toxicity or the carcinogenicity of AMOZ. Nitrofurantoin induced an increase mainly in benign tumours in mice and rats, but in male rats a few malignant tumours were found. Based on these observations, the CONTAM Panel concluded that there is limited evidence that nitrofurantoin is carcinogenic in rats. No information on the chronic toxicity or the carcinogenicity of AHD was identified. Nitrofurazone increased the incidence of mainly benign tumours in mice and rats following oral administration. In male rats a non-dose related increase in carcinomas of the preputial gland was observed. The CONTAM Panel concluded that there is no evidence for the carcinogenicity of nitrofurazone in mice, and that evidence for its carcinogenicity in rats is equivocal. Non-neoplastic effects of nitrofurazone were observed in a chronic toxicity study at the lowest dose tested of 14 mg/kg b.w. per day in mice (ovarian atrophy in females and reduced survival in males) and the lowest dose tested of about 11 mg/kg b.w. per day in rats (testes degeneration). SEM increased the incidence of malignant lung tumours, particularly in female mice. In rats, no increase in tumour incidence was found. The CONTAM Panel concluded that there is limited evidence that SEM is carcinogenic in mice, but not in rats. Based on effects on bones observed in a chronic toxicity study in male rats, a NOAEL of 0.6 mg/kg per day was derived for non-neoplastic effects of SEM. For nifursol the available chronic toxicity studies in rats and dogs did not show clear indication for carcinogenicity. The toxicological information was too limited to derive a NOAEL for non-neoplastic effects of nifursol. No information on the chronic toxicity or the carcinogenicity of DNSH was identified.

In relation to the mode of action, reduction of the nitro-group seems to be the key metabolic pathway leading to reactive intermediates, including reactive oxygen species. Reactive metabolites are capable of binding to proteins and to DNA, being thereby responsible for most of the adverse effects resulting from exposure to nitrofurans. Only for AOZ information was identified regarding the mode of action of the nitrofuran marker metabolites. AOZ plays a role in the inhibition of monoamine-oxidase in animals treated with furazolidone. This may result in an increased susceptibility to neurotoxic effects of certain biogenic amines such as tyramine. Protein binding of reactive nitrofuran metabolites may play a role in the irreversible inhibition of the pyruvate dehydrogenase complex, another potential mechanism underlying neurotoxic effects of nitrofurans, such as polyneuritis.

In human studies, oral administration of furazolidone and nitrofurantoin may lead to a range of adverse reactions, particularly nausea, vomiting and abdominal pain. Both drugs have also been associated with haemolytic anaemia observed in patients deficient in glucose-6-phosphate dehydrogenase. The topical use of nitrofurazone may lead to allergic reactions. Epidemiological studies are reported only for patients treated with nitrofurantoin, and associations were found for cancers of the nervous system in adults, for drug-induced liver injury, and for increased risk of pulmonary adverse events in patients with renal impairment.

Because most of the nitrofurans and their marker metabolites are genotoxic and/or carcinogenic, derivation of health-based guidance values (HBGVs) is not appropriate.

In the case of furazolidone, a lower 95 % confidence limit for a benchmark response of 10 % extra risk ($BMDL_{10}$) value for bronchial adenocarcinomas in mice of 3.5 mg/kg b.w. per day (1.6 mg/kg b.w. per day, expressed as AOZ) was selected as a reference point for carcinogenic effects. Non-neoplastic effects of furazolidone and AOZ were found on red blood cell parameters and enzymes in blood. The lowest BMDL was estimated for the effect of AOZ on alkaline phosphatase (ALP) ($BMDL_{05}$ of 0.02 mg/kg b.w. per day). The CONTAM Panel concluded that this value can be used as reference point for the risk characterisation for non-neoplastic effects.

For furaltadone, the CONTAM Panel concluded that the available data do not provide a suitable basis for deriving a reference point. For AMOZ there is no information on carcinogenicity, and the limited available data indicate that it is non-genotoxic *in vitro*. Therefore, the CONTAM Panel concluded that the risk for carcinogenicity cannot be assessed. There is no information on non-neoplastic effects of furaltadone or AMOZ that could be used for the derivation of a reference point.

In the case of nitrofurantoin, a $BMDL_{10}$ value for osteosarcomas in male rats of 61 mg/kg b.w. per day (29.5 mg/kg b.w. per day, expressed as AHD) was selected as a reference point for carcinogenic effects. For non-neoplastic effects, the most sensitive endpoint for nitrofurantoin is impaired spermatogenesis, but the available data did not allow for a BMD analysis or the derivation of a NOAEL. Effects were observed at the lowest dose tested of 10 mg/kg b.w. per day (4.8 mg/kg b.w. per day, expressed as AHD) and this was selected as a reference point for non-neoplastic effects. The CONTAM Panel noted that the effects at this dose are substantial.

For nitrofurazone, no conclusion could be drawn on its possible carcinogenicity and in the case of SEM, the available information was not suitable to derive a reference point for carcinogenic effects. Non-neoplastic effects of nitrofurazone were found on the testes and the epididymis in rats, while for SEM effects on bone development were observed. The lowest BMDL was estimated for the effect of SEM on bone development ($BMDL_{10}$ of 1.0 mg/kg b.w.). The CONTAM Panel concluded that this value can be used as reference point for the risk characterisation for non-neoplastic effects.

While nifursol is genotoxic *in vitro*, there is no clear indication that it is carcinogenic and for DNSH there is no information on mutagenicity/genotoxicity or carcinogenicity. For non-neoplastic effects, a $BMDL_{05}$ value for the effect of nifursol on liver weight of 11 mg/kg b.w. per day (7.3 mg/kg b.w. per day, expressed as DNSH) was selected as reference point.

Since different critical effects are observed for the different marker metabolites, the CONTAM Panel characterised the risk for each marker metabolite separately. For the actual exposure to nitrofuran marker metabolites, no reliable human dietary exposure assessment could be carried out and, therefore, the CONTAM Panel could not characterise the risk.

To evaluate whether the RPA for nitrofuran metabolites in food of animal origin is adequate to protect public health, the CONTAM Panel considered the scenario in which foods of animal origin, excluding milk and dairy products, are considered to contain one nitrofuran marker metabolite at a concentration level equal to the RPA of 1 µg/kg.

For AOZ, median chronic dietary exposure across dietary surveys for the average consumer would result in a margin of exposure (MOE) for carcinogenicity of about 2.9×10^5 for toddlers and 6.2×10^5 for adults and an MOE for non-neoplastic effects of about 3.6×10^3 for toddlers and 7.7×10^3 for adults. The CONTAM Panel considered that for AOZ these MOEs for carcinogenicity and non-neoplastic effects are sufficiently large and do not indicate a health concern.

For AMOZ, the CONTAM Panel could not conclude on the carcinogenicity. Given that there are no clear indications that furaltadone is more potent than furazolidone with respect to the induction of mammary adenocarcinomas, the CONTAM Panel concluded that the cancer risk from AMOZ, if any, would not be greater than that from AOZ and hence does not indicate a health concern. The CONTAM Panel could not identify a reference point for non-neoplastic effects for AMOZ.

For AHD, median chronic dietary exposure across dietary surveys for the average consumer would result in an MOE for carcinogenicity of about 5.4×10^6 for toddlers and 1.1×10^7 for adults and an MOE for non-neoplastic effects of about 8.7×10^5 for toddlers and 1.8×10^6 for adults. The CONTAM Panel considered that for AHD these MOEs for carcinogenicity and non-neoplastic effects are sufficiently large and do not indicate a health concern.

For SEM the cancer risk could not be assessed. For non-neoplastic effects, median chronic dietary exposure across dietary surveys for the average consumer would result in an MOE of about 1.8×10^5 for toddlers and 3.8×10^5 for adults. The CONTAM Panel considered that for SEM these MOEs for non-neoplastic effects are sufficiently large and do not indicate a health concern.

For DNSH, median chronic dietary exposure across dietary surveys for the average consumer would result in an MOE for non-neoplastic effects of about 1.3×10^6 for toddlers and 2.8×10^6 for adults.

The CONTAM Panel considered that for DNSH these MOEs for non-neoplastic effects are sufficiently large and do not indicate a health concern.

To assess the appropriateness of applying the RPA that is considered adequate to protect public health to other commodities than food of animal origin, the CONTAM Panel considered the scenario in which foods of animal origin, including only those milk and dairy products for which carrageenan is authorised as an additive, and foods of non-animal origin for which carrageenan is authorised as an additive, are considered to be contaminated with SEM at a concentration level equal to the RPA of 1 µg/kg.

AOZ, AMOZ, AHD or DNSH have not been reported to occur in foods of non-animal origin. Only SEM is reported to occur in food of non-animal origin due to its potential presence in the food additive carrageenan, which is used in a large variety of foods. The food additive carrageenan may also be used in foods of animal origin. For SEM, the cancer risk could not be assessed. For non-neoplastic effects, median chronic dietary exposure across dietary surveys for the average consumer would result in an MOE of about 3.4×10^4 for toddlers and 1.0×10^5 for adults. The CONTAM Panel considered that for SEM these MOEs for non-neoplastic effects are sufficiently large and do not indicate a health concern.

The CONTAM Panel recommends that there is need for a carcinogenicity study on SEM according to the current guidelines and that there is need for information on the mechanisms underlying the genotoxic and carcinogenic effects of SEM.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Nitrofurans are synthetic broad-spectrum antimicrobial agents used in some countries in human and veterinary medicine. However, nitrofurans have been prohibited from use in food-producing animals in most countries due to public health and safety concerns, particularly in relation to the carcinogenic potential of either the parent compounds or their metabolites.

In the European Union, nitrofurans were allowed for use in veterinary medicinal products[4] until 1 July 1993, when all nitrofurans were classified as prohibited substances with the exception of furazolidone. This remained the case until 1 July 1995, when furazolidone was also reclassified as a prohibited substance.

Nitrofurans have been evaluated on several occasions by the European Medicines Agency (EMA) and Joint FAO/WHO Expert Committee on Food Additives (JECFA). EMA proposed that nitrofurans[5] (excluding furazolidone) be classified as 'prohibited substances' as there was insufficient information related to mutagenicity and carcinogenicity, while for furazolidone,[6] EMA proposed to classify it as a prohibited substance due to evidence of mutagenicity and carcinogenicity. At its 40th session, JECFA concluded that nitrofurazone was carcinogenic but not genotoxic whereas furazolidone was carcinogenic and genotoxic.

A minimum required performance limit (MRPL) for nitrofurans is set in European Union legislation[7] for the metabolites of furazolidone, furaltadone, nitrofurantoin and nitrofurazone for poultry meat and aquaculture products at the level of 1 µg/kg for all metabolites.

Analytically, residues are checked only for marker metabolites of the 4 nitrofuran chemicals, in particular: 3-amino-2-oxazolidinone (AOZ) for furazolidone, 3-amino-5-methylmorpholino-2-oxazolidinone (AMOZ) for furaltadone, 1-aminohydantoin (AHD) for nitrofurantoin and semicarbazide (SEM) for nitrofurazone.

By virtue of Commission Decision 2005/34/EC,[8] the MRPL is applicable as a reference point for action (RPA) in products of animal origin imported from third countries irrespective of the matrix tested: all food of animal origin containing residues[9] (at or above the RPA of 1 µg/kg is considered non-compliant and removed from the food chain (destruction, re-dispatch, recall). Confirmed findings below the RPA, indicating a recurrent pattern, also trigger specific actions directed towards the third countries of origin.

A similar approach,[10] including possible enforcement actions, applies to food of animal origin produced within the Union, as laid down in Directive 96/23/EC. The two above provisions are confirmed by Regulation (EC) No 470/2009.

As regards SEM, it has repeatedly been demonstrated or claimed that its presence can be caused by other sources than nitrofurazone treatments. Its presence in packaged food has been attributed in the

[4] Nitrofurans were classified as 'All substances belonging to the nitrofuran group' with marker residue 'All residues with the intact 5 nitro structure' for all food-producing animals with a maximum residue limit (MRL) of 5 µg/kg for the target tissues muscle, liver, kidney and fat. The MRL applied to the total residues for all substances within this group.

[5] Nitrofurans Summary Report—Committee for Veterinary Medicinal Products. Available online: http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015183.pdf

[6] Furazolidone summary report—Committee for Veterinary Medicinal Products. Available online: http://www.ema.europa.eu/docs/en_GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500014332.pdf

[7] Commission Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. OJ L 221, 17.8.2002, p. 8.

[8] Commission Decision 2005/34/EC laying down harmonised standards for the testing for certain residues in products of animal origin imported from third countries. OJ L 1, 20.1.2005, p. 6.

[9] Expressed as the sum of the four nitrofurans' marker metabolites.

[10] SANCO -E.2(04)D/521927. Available online: http://ec.europa.eu/food/committees/regulatory/scfcah/controls_imports/summary35_en.pdf

past to the use of azodicarbonamide as a blowing agent used to foam the plastic gaskets in the metal lids of jars and bottles. However, this use of azodicarbonamide is no longer permitted in the EU. Presence of SEM could also be possible due to the use of azodicarbonamide as a flour treatment agent (dough improver) in bread production, however, such use is also not permitted in the EU. SEM can also result as a reaction product of hypochlorite with some food additives (e.g. carrageenan) with some foods (such as egg white powder). Natural background levels, formation during drying of certain foods, as well as unidentified sources are often cited as possible reason for detection of SEM in food commodities (e.g. certain crayfish, seaweed, eggs, whey and certain varieties of honey).

In analysis of food of animal origin, this has led – where possible – to the introduction of washing steps in the analytical techniques in order to detect only tissue bound molecules, as only these are considered indicative for illegal treatment.

Findings of nitrofurans

From 2000 onwards, nitrofurans have been the subject of more than 700 messages in the Rapid Alert System for Food and Feed. For the different marker metabolites, reported levels ranged from 0.1–1 200 µg/kg for AOZ (282 messages), 0.3–140 µg/kg for AMOZ (97 messages), 0.3–40 µg/kg for AHD (6 messages) and from 0.37–7 500 µg/kg for SEM (351 messages).

Commodities reported as containing residues of nitrofurans were: crustaceans and products thereof (482), poultry meat and poultry meat products (150), fish and fish products (54), meat other than poultry and derived products (46), honey and royal jelly (20), eggs and egg products (13), food additives and flavourings (2) and prepared dishes and snacks (1).

Safeguard measures[11] have been adopted for a number of food commodities originating from several third countries. Only once the import checks have demonstrated that all consignments are compliant the safeguard measures could be lifted or no longer prolonged.

Article 19 (2) of Regulation (EC) No 470/2009 states that the Commission shall, where appropriate, submit a request to EFSA for a risk assessment as to whether the reference points for action are adequate to protect human health.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission requests EFSA in accordance with Article 29 of Regulation (EC) No 178/2002 for a scientific opinion on the risks to human health related to the presence of nitrofurans and their metabolites in food.

In particular this opinion should comprise the:

a) evaluation of the toxicity of nitrofurans and their metabolites for humans, considering all relevant toxicological endpoints and identification of the toxicological relevance of nitrofurans and their metabolites present in food;

b) exposure of the EU population to nitrofurans and their metabolites from food, including the consumption patterns of specific (vulnerable) groups of the population;

c) assessment of the appropriateness of using marker metabolites of nitrofurans for the reference point for action for food of animal origin;

[11] For example: Commission Decision 2008/630/EC on emergency measures applicable to crustaceous imported from Bangladesh and intended for human consumption (OJ L 205, 1.8.2008, p. 49); Commission Decision 2002/994/EC concerning certain protective measures with regard to the products of animal origin imported from China (OJ L 348, 21.12.2002, p. 154); Commission Decision 2010/381/EU on emergency measures applicable to consignments of aquaculture products imported from India and intended for human consumption (OJ L 174, 9.7.2010, p. 51).

d) evaluation whether a reference point for action of 1 µg/kg for nitrofuran metabolites as defined in legislation in food of animal origin is adequate to protect public health;

e) assessment of the appropriateness of applying the reference point for action considered adequate to protect public health to other commodities than food of animal origin.

ASSESSMENT

1. Introduction

Nitrofurans are synthetic chemotherapeutic agents with a broad antimicrobial spectrum, including Gram-positive and Gram-negative bacteria and protozoa. Nitrofurans are bacteriostatic but, at high doses, their action may also be bactericidal. Structurally, the essential component of nitrofurans is a furan ring with a nitro-group, and the latter is a requisite for antimicrobial activity. Nitrofurans are very effective antimicrobial agents that, prior to their prohibition for use in food-producing animals in the European Union (EU), were widely used in livestock (cattle, pigs and poultry), aquaculture and bees.

The nitrofurans considered in this opinion are **furazolidone**, **furaltadone**, **nitrofurantoin**, **nitrofurazone** (also known as nitrofural or Furacilin) and **nifursol**. In the case of furazolidone, furaltadone, nitrofurantoin and nitrofurazone, these are the nitrofurans specifically listed in Annex II to Commission Decision 2002/657/EC[12] for the metabolites for which a minimum required performance limit (MRPL) of 1 µg/kg is specified. Nifursol is also included in this opinion because of its former widespread use as an additive in feedingstuffs for turkeys for the prevention of 'blackhead disease' (histomoniasis).

In human medicine, furazolidone, nitrofurantoin and nitrofurazone are still used (see Section 1.3.1). In veterinary medicine, nitrofurans are no longer authorised for use in food-producing animals in the EU because no acceptable daily intake (ADI) could be established owing to positive results in genotoxicity testing. Nitrofurans are also not allowed to be used in food-producing animals in countries such as the USA, Australia, the Philippines, Thailand and Brazil.

Nitrofurans share a nitrofuran ring but have different side-chains (such as 3-amino-2-oxazolidinone in the case of furazolidone), connected via a so-called azomethine bond. A characteristic of nitrofurans is the short half-life of the parent compounds due to extensive metabolism, primarily a reduction of the nitro-group, such that they do not occur generally as residues in foods of animal origin. This nitroreduction results in the formation of reactive metabolites able to bind covalently to tissue macromolecules, including proteins. In food-producing animals, these metabolites have a relatively long half-life. When such animal tissues are consumed as food, side-chains may be released from these metabolites under the acidic conditions of the human stomach, namely 3-amino-2-oxazolidinone (AOZ) from the metabolites of furazolidone, 3-amino-5-methylmorpholino-2-oxazolidinone (AMOZ) from the metabolites of furaltadone, 1-aminohydantoin (AHD) from the metabolites of nitrofurantoin, semicarbazide (SEM) from the metabolites of nitrofurazone and 3,5-dinitrosalicylic acid hydrazide (DNSH) from the metabolites of nifursol. These released side-chains of nitrofuran metabolites have the potential to be carcinogenic and mutagenic. In principle, the side-chains can also be released during acid hydrolysis from the parent compounds and other metabolites. This implies that the side-chains are potential metabolites themselves following hydrolysis of the parent compound in the stomach, but this has been demonstrated only for pigs treated with furazolidone. Free AOZ was also detected in rats fed with protein-bound residues of furazolidone. The side-chains are also excellent marker metabolites for the presence of protein-bound residues following sample treatment with acid and derivatisation with nitrobenzaldehyde.

The European Food Safety Authority (EFSA) scientific opinion entitled '*Guidance on methodological principles and scientific methods to be taken into account when establishing Reference Points for Action (RPAs) for non-allowed pharmacologically active substances present in food of animal origin*' (EFSA CONTAM Panel, 2013) identified an approach based on both analytical and toxicological considerations for establishing RPAs for various categories of non-allowed pharmacologically active substances. However, the opinion also identified certain categories of non-allowed pharmacologically active substances for which toxicological screening values based on the procedure described might not

[12] Commission Decision 2002/657/EC implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. OJ L 221, 17.8.2002, p. 8–36.

be sufficiently health protective and such substances are considered to be outside the scope of the procedure. Such substances include those causing blood dyscrasias (such as aplastic anaemia) or allergy, or which are high-potency carcinogens. As the side-chains of nitrofurans are hydrazines and are as such considered as potential high-potency carcinogens, a specific risk assessment is required.

The scope of this opinion is primarily directed at nitrofurans and their metabolites, in accordance with the Commission request 'for a scientific opinion on the risks to human health related to the presence of nitrofurans and their metabolites in food'. However, to adequately address the issue of 'assessment of the appropriateness of applying the reference point for action considered adequate to protect public health to other commodities than food of animal origin' (see Terms of Reference), consideration of the potential occurrence of SEM in food, from a variety of sources other than as a metabolite of nitrofurazone, is included in the opinion (Appendix A). For this purpose, the concentrations of SEM in food and dietary exposure calculated by the EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC Panel) in its opinion on SEM (EFSA, 2005)—updated by changes in regulations such as the subsequent prohibition of certain uses of azodicarbonamide in food and new information on SEM, particularly relating to its occurrence in food products from use of the food additive carrageenan—are considered.

1.1. Previous assessments

Nitrofurans have been the subject of several previous assessments by international, European and national organisations.

1.1.1. International and European agencies

At its 40th meeting, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated furazolidone and nitrofurazone.

Based on the positive results of **furazolidone** in genotoxicity tests *in vitro* and the increased incidence of malignant tumours in mice and rats, JECFA concluded that furazolidone is a substance that is genotoxic and carcinogenic. Owing to the genotoxic and carcinogenic nature of furazolidone, and the lack of sufficient data on the nature and toxic potential of the bound residues, JECFA was unable to establish an ADI (FAO/WHO, 1993a). As a result, JECFA could not recommend a maximum residue limit (MRL). The residue data were insufficient to identify a marker residue and insufficient information was available on the quantity and nature of the total residues (FAO/WHO, 1993c, e).

Nitrofurazone caused benign tumours that were restricted to endocrine organs and the mammary gland. Nitrofurazone is genotoxic *in vitro* but not *in vivo*. From these data, JECFA concluded that nitrofurazone is a secondary carcinogen causing effects in endocrine-responsive organs by a mechanism that remains to be elucidated. Effects on steroidogenesis may be involved in the process of tumour development. No ADI could be established because a no-effect level had not been identified for the tumorigenic effects. JECFA noted that the lowest dose tested of 11 mg/kg body weight (b.w.) per day caused a high incidence of testicular degeneration in a 2-year carcinogenicity study. Moreover, no study on reproductive performance was available. A no-effect level could also not be identified for the degenerative changes in the joints of rats (FAO/WHO, 1993b). JECFA could not recommend an MRL because no ADI was established. Furthermore, the residue data were insufficient to identify a marker residue and insufficient information was available on the quantity and nature of the total residues (FAO/WHO, 1993d, f).

The International Agency for Research on Cancer (IARC) evaluated furazolidone in 1983, furaltadone in 1974, nitrofurantoin in 1990, nitrofurazone in 1974 and 1990, and SEM hydrochloride in 1976. Data on the carcinogenicity of **furazolidone** in experimental animals were not available for evaluation. In the absence of epidemiological data, no evaluation of the carcinogenicity of furazolidone in humans could be made and the IARC concluded that furazolidone is not classifiable as regards its carcinogenicity to humans (group 3) (IARC, 1983, 1987). **Furaltadone** caused mammary carcinomas and lymphoblastic lymphomas in rats following oral administration of its hydrochloride.

No case reports or epidemiological studies were available (IARC, 1974). Based on this information, the IARC concluded that furaltadone is possibly carcinogenic to humans (group 2B) (IARC, 1987). For the evaluation of the carcinogenicity of **nitrofurantoin**, only limited evidence was available in experimental animals and inadequate evidence in humans. The IARC concluded that nitrofurantoin is not classifiable as to its carcinogenicity in humans (group 3) (IARC, 1990a). During its most recent evaluation of **nitrofurazone**, the IARC concluded that only limited evidence was available for its carcinogenicity in experimental animals and inadequate evidence in humans. The IARC concluded that nitrofurazone is not classifiable as to its carcinogenicity in humans (group 3) (IARC, 1990b). SEM hydrochloride caused angiomas, angiosarcomas and lung tumours in mice after oral administration. Therefore, the IARC concluded that SEM hydrochloride is carcinogenic in mice after oral administration. No human data (case reports or epidemiological studies) were available (IARC, 1976). The IARC concluded that SEM hydrochloride is not classifiable as regards its carcinogenicity in humans (group 3), as no adequate data were available for humans and limited evidence was available for experimental animals (IARC, 1987).

The Scientific Committee on Animal Nutrition (SCAN) evaluated the use of **furazolidone**, **nitrofurazone** and **bifuran** (furazolidone + nitrofurazone) in feedingstuffs (SCAN, 1977). The Committee identified numerous data gaps concerning methods of analysis, metabolism, carcinogenicity and mutagenicity. It was concluded that, in the absence of additional data, the use of furazolidone, nitrofurazone and bifuran as feed additives should be prohibited.

In 1982, the SCAN evaluated the use of **nifursol** in feedingstuffs for turkeys. Nifursol showed some hepatotoxic effects in a chronic feeding study in rats, but no carcinogenicity was observed. In rats, no reproductive toxicity was observed in a three-generation study. From these long-term studies, a no-effect level of 400 mg/kg feed was identified. Mutagenicity studies in several strains of *Salmonella enterica* subsp. *enterica* serovar Typhimurium were negative. No embryotoxicity/teratology studies were available. Fertility and hatchability of eggs were not affected by a 4-month exposure to 75 mg nifursol/kg feed. Based on the available information, the Committee concluded that the use of nifursol as an additive in feedingstuffs for turkeys at a level of 50–75 mg/kg could be maintained, subject to a withdrawal period of 5 days before slaughter (SCAN, 1982). In 2001, the use of nifursol as a feed additive was re-evaluated. Based on the available data, no conclusion could be drawn regarding the genotoxicity of nifursol. The available data did not give a clear indication of any tumorigenicity from nifursol. However, owing to the shortcomings of the study and limited reporting, the Committee indicated that this conclusion should be regarded as provisional. In addition, the Committee noted the non-conclusive results of a chronic toxicity study in dogs, the lack of data on developmental toxicity and that only one metabolic route is common between turkeys and rats. It was concluded that no ADI could be established. The human exposure to nifursol residues (including metabolites) could not be determined because of a lack of data. Overall, it was concluded that the safety of nifursol for the human consumer cannot be ensured (SCAN, 2001). In 2003, additional studies on mutagenicity and residues became available. However, the data did not allow the Committee to conclude that nifursol is non-genotoxic *in vivo*. It was reiterated that no ADI could be established. The new residue studies did not allow the human exposure to nifursol residues (including metabolites) to be determined. The SCAN reiterated the conclusion that the safety of nifursol for human consumers cannot be ensured (SCAN, 2003).

The Committee for Veterinary Medicinal Products (CVMP) of the European Agency for the Evaluation of Medicinal Products (EMEA; now the European Medicines Agency (EMA)) published an evaluation of nitrofurans in 1996. Owing to the lack of sufficient data for **nitrofurazone**, **nitrofurantoin** and **furaltadone**, the CVMP recommended that these nitrofurans be included in Annex IV of council regulation (EEC) No 2377/90, which is the 'list of pharmacologically active substances for which no maximum levels can be fixed'. Because industry was planning further toxicological studies for furazolidone, the provisional MRL was retained until the following evaluation (EMA, 1996). After this evaluation, new data on mutagenicity, subchronic toxicity, residue depletion, bioavailability of residues and residue analysis were submitted by industry for **furazolidone**. Based on this new information, the CVMP concluded that a no-observed-effect-level

(NOEL) could not be established and that AOZ is mutagenic in all investigated test systems. It was noted that furazolidone itself is mutagenic and carcinogenic in mice and rats. Total residues were in the mg/kg range in all edible tissues. Bound residues were shown to be bioavailable in rats fed with meat from furazolidone-treated pigs that were slaughtered 45 days after the last treatment. AOZ could be released from the bound residues in pig liver, even after 45 days. Therefore, the CVMP proposed that furazolidone also be included in Annex IV of Council Regulation (EEC) No 2377/90 (EMA, 1997).

SEM can be present in food from different sources (see Section 3.3 and Appendix A). The AFC Panel issued preliminary advice on SEM in packaged foods in July and October 2003. In 2005, the AFC Panel assessed the risk posed by SEM in all types of food. The AFC Panel concluded that SEM is mutagenic but not clastogenic in some test systems *in vitro*, notably in the absence of an exogenous metabolising system. However, the weak genotoxicity exerted by SEM *in vitro* is not expressed *in vivo*. SEM has been shown to be carcinogenic in mice, but not in rats. The AFC Panel concluded that SEM is a weak non-genotoxic carcinogen for which a threshold mechanism can be assumed. A large margin of at least five orders of magnitude exists between the dose causing tumours in experimental animals and human exposure. The AFC Panel therefore concluded that the issue of carcinogenicity is not of concern for human health at the concentrations of SEM encountered in food (EFSA, 2005).

1.1.2. National agencies

In 2002, the German Federal Institute for Consumer Health Protection and Veterinary Medicine (BgVV) evaluated the findings of positive nitrofuran metabolites in poultry, shrimps and rabbits. In its statement, BgVV concluded that, based on the available data, an estimation of human dietary exposure to nitrofuran metabolites was not feasible. In addition, a no-observed-adverse-effect level (NOAEL) could not be established and information on dose–response relationships was insufficient. Therefore, BgVV could not perform a risk assessment; however, it stated that a health risk, especially through repeated consumption of food containing nitrofuran metabolites, cannot be excluded (BgVV, 2002).

The National Institute for Public Health and Environment (RIVM; Rijksinstituut voor Volksgezondheid en Milieu) in 2003 evaluated the risk of **furazolidone** occurrence in shrimps. AOZ had been detected in shrimps at a concentration of 5 µg/kg. The RIVM concluded that furazolidone is genotoxic and carcinogenic and that, therefore, no ADI could be established. AOZ is genotoxic, but no carcinogenicity studies were available. However, it was assumed that AOZ is involved in the carcinogenicity of furazolidone and that, as such, AOZ is also genotoxic and carcinogenic. Based on tumour incidences in rats and mice reported by JECFA (FAO/WHO, 1993a), a virtual safe dose (VSD)[13] of 50 ng/kg b.w. per day was derived. Because AOZ and not furazolidone was analysed in the shrimp samples, the ratio of the molecular weights of AOZ and furazolidone (2.2) was used to convert the AOZ concentration of 5 µg/kg into the furazolidone concentration of 11 µg/kg. Based on a mean shrimp consumption of 8.4 g per week, the exposure was estimated to be 0.22 ng furazolidone/kg b.w. per day for a 60-kg person. The margin of safety between the exposure and the VSD was about 200, and the risk to public health of such an exposure was considered nil (RIVM, 2003).

Food Standards Australia New Zealand (FSANZ) in 2004 assessed the risk of nitrofurans in prawns. It was noted that furazolidone induces malignant tumours in rats at doses of 25 mg/kg b.w. per day. Therefore, furazolidone was considered a potential carcinogen in humans. However, insufficient data were available to conclude that tumour formation is initiated through a genotoxic mechanism and it remained unclear if a threshold mechanism can be assumed. Owing to the lack of data, FSANZ assumed that the toxicity of AOZ is the same as the toxicity of furazolidone. FSANZ estimated the exposure to AOZ from prawns. Based on a mean consumption of prawns of 75 g per day and a high consumption (95th percentile) of 250 g per day, and the lower- (LB) and upper-bound (UB) mean concentrations of AOZ in prawns, dietary exposure was estimated to range between 0.9 and 1.9 ng/kg b.w. per day for consumers of the mean level and between 3.0 and 6.4 ng/kg b.w. per day for high-level consumers. The margin of exposure (MOE) between the dose of furazolidone causing tumours in

[13] The dose estimated to be associated with an additional lifetime cancer risk of 1 in 10^6.

experimental animals and the dietary exposure to AOZ from prawns ranged between 4.2×10^6 and 25×10^6. The risk was also characterised by comparing the dietary exposure with the ADI (0.4 µg/kg b.w.) that had previously been established in Australia. Using the highest exposure calculated, the exposure is 1.5 % of the ADI. The nitrofuran marker metabolites AMOZ, AHD and SEM were not included in the risk assessment because of the low prevalence of these marker metabolites in prawn samples, the lack of toxicological data on furaltadone and AMOZ, and the lower carcinogenic potential of nitrofurazone than of furazolidone. FSANZ concluded that the public health risk from nitrofuran residues in prawns is very low (FSANZ, 2004).

1.2. Chemical characteristics

Furazolidone[14] (3-{(E)-[(5-nitro-2-furyl)methylene]amino}-1,3-oxazolidin-2-one; Chemical Abstracts Service (CAS) No 67-45-8; Figure 1) consists of odourless yellow crystals with the molecular formula $C_8H_7N_3O_5$ and a molecular weight of 225.16 g/mol. It darkens under strong light.

Furazolidone decomposes at 256–257 °C. Its solubility in water at pH 6 is 40 mg/L. The octanol/water partition coefficient (log K_{ow}) is –0.04 and the vapour pressure is 2.6×10^{-6} mmHg at 25 °C. Henry's law constant is estimated to be 3.3×10^{-11} atm-m^3/mol at 25 °C.

Furazolidone can be hydrolysed at low pH to AOZ (3-amino-2-oxazolidinone, $C_3H_6N_2O_2$, molecular weight 102.09 g/mol, Figure 1) (see Section 8.1). However, AOZ, as a side-chain, will also be present in metabolites, including protein-bound residues, from which it can be released by acid treatment. Therefore, AOZ is regarded as the marker residue in food analysis.

Furaltadone[14] (5-(4-morpholinylmethyl)-3-{(E)-[(5-nitro-2-furyl)methylene]amino}-1,3-oxazolidin-2-one; CAS No 139-91-3; Figure 1) consists of odourless yellow crystals with the molecular formula $C_{13}H_{16}N_4O_6$ and a molecular weight of 324.29 g/mol.

Furaltadone decomposes at 206 °C. Its solubility in water is 750 mg/L. The log K_{ow} is 0.25 and the vapour pressure is 2.9×10^{-9} mmHg at 25 °C. Henry's law constant is estimated to be 1.46×10^{-16} atm-m^3/mol at 25 °C.

Furaltadone can be hydrolysed at low pH to AMOZ (3-amino-morpholinomethyl-2-oxazolidinone, $C_8H_{15}N_3O_3$, molecular weight 201.22 g/mol, Figure 1) (see Section 8.1). However, AMOZ, as a side-chain, will also be present in metabolites, including protein-bound residues, from which it can be released by acid treatment. Therefore, AMOZ is regarded as the marker residue in food analysis.

Nitrofurantoin[14] (1-{(E)-[(5-nitro-2-furyl)methylene]amino}-2,4-imidazolidinedione; CAS No 67-20-9; Figure 1) consists of orange-yellow needles with the molecular formula $C_8H_6N_4O_5$ and a molecular weight of 238.16 g/mol.

Nitrofurantoin decomposes at 270–272 °C. Its solubility in water is 80 mg/L. The log K_{ow} is –0.47 and the vapour pressure is 2.8×10^{-10} mmHg at 25 °C. Henry's law constant is estimated to be 1.33×10^{-12} atm-m^3/mol at 25 °C.

Nitrofurantoin can be hydrolysed at low pH to AHD (1-aminohydantoin, $C_3H_5N_3O_2$, molecular weight 115.09 g/mol, Figure 1) (see Section 8.1). However, AHD, as a side-chain, will also be present in metabolites, including protein-bound residues, from which it can be released by acid treatment. Therefore, AHD is regarded as the marker residue in food analysis.

Nitrofurazone[14] (2-(E)-[(5-nitro-2-furyl)methylene]hydrazinecarboxamide; CAS No 59-87-0; Figure 1) consists of pale-yellow needles with the molecular formula $C_6H_6N_4O_4$ and a molecular weight of 198.14 g/mol. It darkens after prolonged exposure to light.

[14] The main chemical characteristics were taken from the Merck Index, ChemSpider and the Toxnet databases ChemIDplus and HSDB.

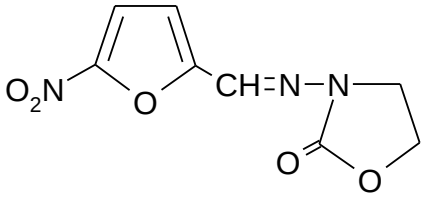
Nitrofurazone decomposes at 236–240 °C. Its solubility in water is 210 mg/L. The log K_{ow} is 0.23 and the vapour pressure is 4.31×10^{-6} mmHg at 25 °C. Henry's law constant is estimated to be 3.1×10^{-13} atm-m^3/mol at 25 °C.

Nitrofurazone can be hydrolysed at low pH to SEM (semicarbazide, CH_5N_3O, molecular weight 75.08 g/mol, Figure 1) (see Section 8.1). However, SEM, as a side-chain, will also be present in metabolites, including protein-bound residues, from which it can be released by acid treatment. Therefore, SEM is regarded as the marker residue in food analysis.

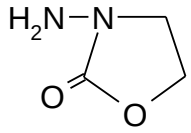
Nifursol[14] (2-hydroxy-3,5-dinitro-N′-[(E)-(5-nitro-2-furyl)methylene]benzohydrazide; CAS No 16915-70-1; Figure 1) is a yellow solid with the molecular formula $C_{12}H_7N_5O_9$ and a molecular weight of 365.21 g/mol.

Nifursol decomposes at 215–220 °C. The log K_{ow} is estimated to be 2.48. Using this value, the water solubility was estimated to be 38 mg/L. Henry's law constant is estimated to be 1.21×10^{-14} atm-m^3/mol at 25 °C (EPISuite, ChemSpider).

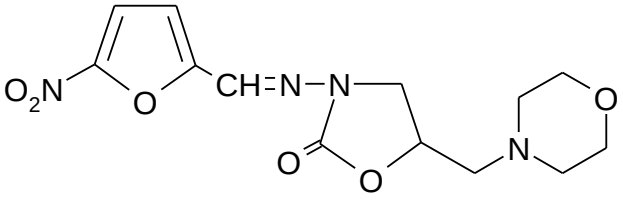
Nifursol can be hydrolysed at low pH to DNSH (3,5-dinitrosalicylic acid hydrazide, $C_7H_6N_4O_6$, molecular weight 242.15 g/mol, Figure 1) (see Section 8.1). However, DNSH, as a side-chain, will also be present in metabolites, including protein-bound residues, from which it can be released by acid treatment. Therefore, DNSH is regarded as the marker residue in food analysis.



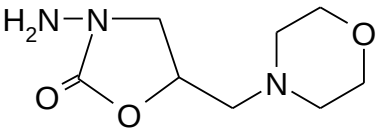
Furazolidone



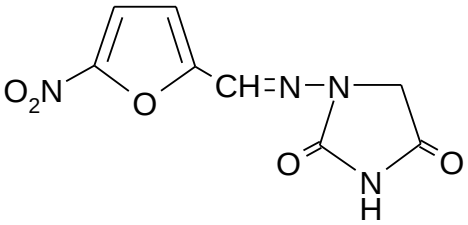
3-amino-2-oxazolidinone (AOZ)



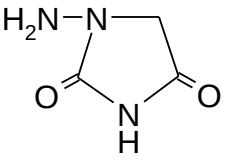
Furaltadone



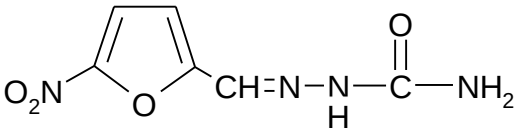
3-amino-5-methylmorpholino-2-oxazolidinone (AMOZ)



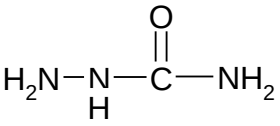
Nitrofurantoin



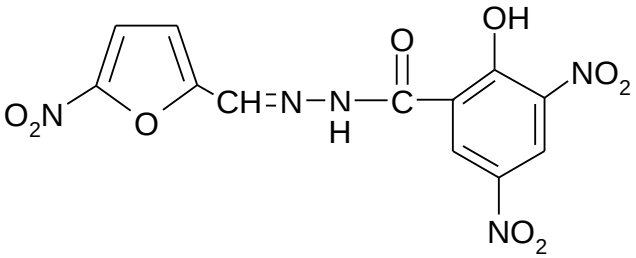
1-aminohydantoin (AHD)



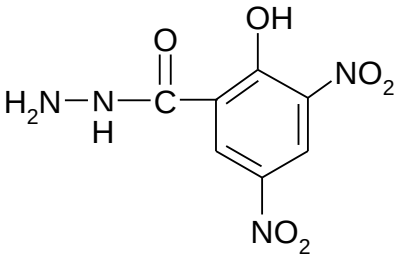
Nitrofurazone



semicarbazide (SEM)



Nifursol



3, 5-dinitrosalicylic acid hydrazide (DNSH)

Figure 1: Chemical structures of the nitrofurans considered in this opinion and their marker metabolites

1.3. Therapeutic use of nitrofurans

1.3.1. Therapeutic use of nitrofurans in humans

Furazolidone has antiprotozoal and antibacterial activity. Furazolidone is bactericidal and appears to act by interfering with bacterial enzyme systems. Resistance is reported to be limited. It is used in the treatment of giardiasis and cholera. Owing to its low cost and low rate of primary *Helicobacter pylori* resistance, the World Gastroenterology Organisation recommends its use against *H. pylori* infections in developing countries (Hunt et al., 2011; Zullo et al., 2012). Furazolidone is available in some EU countries. It may be given orally at a dose of 100 mg four times daily (EMA personal communication, 2015). It is usually given for 2 to 5 days. For the treatment of giardiasis, cholera and other diarrhoeal diseases caused by susceptible organisms, children and infants from 1 month of age might be given oral furazolidone at a dose of 1.25 to 1.5 mg/kg b.w. four times daily for 2 to 5 days normally (Brayfield, 2014).

Furaltadone was formerly given orally as an antibacterial agent but was later withdrawn owing to its toxic effects (Brayfield, 2014).

Nitrofurantoin is used in the treatment of uncomplicated lower urinary tract infections, including prophylaxis, or for long-term suppressive therapy in recurrent infection. It is given orally at a usual dose of 50 to 100 mg four times daily (Brayfield, 2014).

In the past, formulations were used with micro- and/or macrocrystalline nitrofurantoin (see also Section 8.1.2; Cunha, 1988; D'Arcy, 1985; Brumfitt and Hamilton-Miller, 1998). Nowadays, macrocrystalline nitrofurantoin is mostly used. A common long-term prophylactic dose is 50 to 100 mg at bedtime (Brayfield, 2014).

Contraindications

Nitrofurantoin should not be given to patients with renal impairment, as antibacterial concentrations in the urine may not be attained and toxic concentrations in the plasma can occur. Nitrofurantoin is also contraindicated in patients known to be hypersensitive to nitrofurans, in those with glucose-6-phosphate dehydrogenase (G6PD) deficiency, and in infants (in the UK it is contraindicated below 3 months of age, although the USA permits use from 1 month of age) (Brayfield, 2014).

Nitrofurazone has a broad spectrum of antibacterial and antitrypanosomal activity. Nitrofurazone is used topically for wounds, burns and skin infections. It is usually applied at a concentration of 0.2 % in a water-soluble or water-miscible basis (Brayfield, 2014). Nitrofurazone is also available in ointments and cutaneous powders at a concentration of 0.2 % (2 mg/g) (EMA personal communication, 2015).

Nifursol has not been used in human medicine (Brayfield, 2014).

In short, furazolidone and nitrofurantoin may be used orally. Furazolidone is used for certain gastrointestinal infections and cholera. Nitrofurantoin is mainly used to treat urinary tract infections and for long-term prophylaxis of urinary tract infections. Nitrofurazone is used topically for wounds, burns and skin infections.

1.3.2. Therapeutic use of nitrofurans in livestock, horses and fish

The use of nitrofurans for animal production (including fish) was prohibited in the EU because of concerns about their carcinogenicity and potential harmful effects on human health. The use of nitrofurans in food-producing animals is also not allowed in countries such as the USA, Australia, the Philippines, Thailand and Brazil (Khong et al., 2004). Previously, they had been widely used in the prophylactic and therapeutic treatment of infections caused by bacteria and protozoa mainly affecting

the gastrointestinal or the urinary tracts of swine, cattle, poultry and rabbits. Nifursol was commonly used as a feed additive in turkeys (Vass et al., 2008c).

In certain third countries, nitrofurans may be applied to prevent or control a number of bacterial fish diseases (e.g. edwardsiellosis, vibriosis, branchiomycosis, columnaris and tail rot disease) and bacterial shell disease, appendage rot, septicaemias, bacterial fouling, gill diseases and necrotic hepatopancreas of shrimps (Liao et al., 2000). Limited data are available on the specific types and amounts of antibiotics used in aquaculture. The Food and Agriculture Organization (FAO) have compiled a list of antibiotics that are potentially used in aquaculture facilities throughout the world (26 different antibiotics, including nitrofurans); however, specific data on actual antibiotic usage were not available (FAO, 2005). To investigate global antibiotic usage in aquaculture, Sapkota et al. (2008) compiled country-specific data for the top 15 aquaculture-producing countries during the period 1990–2007, which together accounted for 94 % of global aquaculture production; according to this paper, furazolidone was used in China, the Philippines, Chile, Norway and Taiwan.

Aside from the ban on the use in food-producing species, their therapeutic application has been limited by a number of adverse effects in target species (Huber, 1982).

Furazolidone has been administered to chickens, turkeys and swine for the control of various digestive infections, especially salmonellosis and coccidiosis. Furazolidone has been widely used as an antibacterial and antiprotozoal feed additive for poultry, cattle and farmed fish in China (Hu et al., 2007). For poultry, it was administered in the feed at a concentration of 0.04 % for 10 days, while in large animals it was given orally at doses of 10–12 mg/kg b.w. for 5–7 days (Brander et al., 1991). In a more recent study by Chadfield and Hinton (2003), the inclusion of lower levels of furazolidone (200 mg/kg) in broiler chick feed was unsuccessful in treating already established *Salmonella enterica* subsp. *enterica* serovar Enteritidis infections; by contrast, furazolidone administration at the same dose regime 1 week prior to challenge with the same bacterial strains and continuous dosing for a further week prevented bacterial colonisation of the intestine, liver and spleen. Therapeutic schedules for fish and shrimp diseases were 10 mg/L baths for 1 day or 10 mg/kg b.w. daily by oral administration for 3–6 days (Liao et al., 2000). Furazolidone is well absorbed by fish, and has typically been administered as medicated feed, unlike most nitrofurans, which are poorly absorbed from the gastrointestinal tract (Park et al., 2012). The most used nitrofuran in salmon farming in Norway was furazolidone. The quantity of furazolidone sold annually for treating farmed fish in Norway varied between 0 and 15 840 kg from 1980 to 1993 (the maximum quantity sold was in 1987 (Grave et al., 1990, 1996). From 1994, the use of furazolidone for salmon was prohibited in Norway.

Furaltadone has been primarily used for the treatment of enteric diseases of poultry (salmonellosis, colibacillosis, coccidiosis, histomoniasis) at a dose of 0.02–0.04 % in feed or drinking water for a maximum of 10 days. The drug has also been used for the treatment of mammary infections in dairy cows (500 mg/quarter) and for strangles (equine adenitis) in horses, in which case it was applied systemically (13 mg/kg b.w. per os (p.o. (orally)) for 5 days (Huber, 1982).

Nitrofurantoin has been used by the oral route in calves and horses at daily doses of 10 mg/kg b.w. for the treatment of severe urinary infections (Botsoglou and Fletouris, 2001).

Nitrofurazone has been used locally at a concentration of 0.2 % to treat wounds and diseases of the skin, ear, eye and reproductive tract. Intramammary application has also been used to treat mastitis in dairy cows (Huber, 1982). Like other nitrofurans, it has also been applied by the oral route to treat enteric infections, such as coccidiosis and salmonellosis in poultry and swine, as well as in small ruminants (Robertson, 1982) and for pasteurellosis in rabbits (dosages not found). It has also been widely used as a feed additive, in general at an inclusion rate of 0.05 % in the feed or at 100 mg/head for piglets (Brander et al., 1991).

Finally, **nifursol** is a chemotherapeutic agent that was authorised in the EU as a feed additive for the first time in 1982 for the prevention of histomoniasis in turkeys. The inclusion rate into complete

feedingstuffs was regulated by EU law to be between 50 and 75 mg/kg. The authorisation of nifursol as a feed additive was withdrawn in the EU with effect of 31 March 2003.

2. Legislation

According[15] to Article 3 of Regulation (EC) No 470/2009 of the European Parliament and of the Council,[16] any pharmacologically active substance intended for use in the Union in veterinary medicinal products (VMPs) which are to be administered to food-producing animals shall be subject to an opinion of the EMA on the MRL, formulated by the CVMP. The opinion consists of a scientific risk assessment and risk management recommendations. Pharmacologically active substances, for which the opinion concludes that no MRL is needed or that a (provisional) MRL should be established, are subsequently classified in Table 1, 'allowed substances', of Regulation (EU) 37/2010.[17] All use of other pharmacologically active substances in VMPs is not allowed. A specific group of the non-allowed substances is the group of 'prohibited substances', listed in Table 2 of Regulation (EU) 37/2010. This group of 'prohibited substances' includes, *inter alia*, nitrofurans, without specifying individual substances. In the EU, the application of furaltadone, nitrofurantoin and nitrofurazone to food-producing animals was banned in 1993. The ban on furazolidone followed in 1995. For these nitrofurans, no MRL could be recommended because the available data were not sufficient to allow a safe limit to be identified or because a final conclusion concerning human health with regard to residues of a substance could not be established, given the lack of scientific information.

Article 18 of Regulation (EC) No 470/2009 stipulates that, for substances which are not classified as 'allowed substances' in accordance with that Regulation, an RPA may be established to ensure the functioning of controls for food of animal origin. Food of animal origin containing residues of such substances at or above the RPA is considered not to comply with EU legislation. Until now, RPAs have been based on only analytically driven MRPLs, and no consideration has been given to the toxicological profile of non-allowed substances. The MRPLs for four nitrofuran marker metabolites and a few other prohibited substances are specified in Annex II of Commission Decision 2002/657/EC. For the metabolites of furazolidone, furaltadone, nitrofurantoin and nitrofurazone, an MRPL value of 1 µg/kg each is specified for poultry and aquaculture products. Nifursol is not included in the Annex.

Under the terms of Commission Decision 2005/34/EC,[18] these MRPLs are currently to be used as RPAs, irrespective of the matrix tested, for the purpose of the control of residues when analytical tests are being carried out in the framework of import control. However, this Decision regulated imports from third countries only and did not apply to food produced within the Union. As a number of products of animal origin originating from Member States were found to contain nitrofurans and other prohibited substances below and above the MRPLs, the European Commission (EC) and the Member States agreed to also apply the approach laid down in Decision 2005/34/EC, with the necessary changes, to food of animal origin produced within the Union. This implies, in particular, that the MRPLs set in accordance with Commission Decision 2002/657/EC shall also be used as RPAs. This approach, moreover, means that any detection of substances, the use of which is not authorised in the Union, regardless of the level found, shall be followed by an investigation into the source of the

[15] In this scientific opinion, where reference is made to European legislation (Regulations, Directives, Decisions), the reference should be understood as relating to the most current amendment, unless otherwise stated.

[16] Regulation (EC) No 470/2009 of the European Parliament and of the Council of 6 May 2009 laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No 2377/90 and amending Directive 2001/82/EC of the European Parliament and of the Council and Regulation (EC) No 726/2004 of the European Parliament and of the Council. OJ L 152, 16.6.2009, p. 11–22.

[17] Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. OJ L 15, 20.1.2010, p. 1–72.

[18] Commission Decision 2005/34/EC laying down harmonised standards for the testing for certain residues in products of animal origin imported from third countries. OJ L 16, 20.1.2005, p. 61–63.

substance in question and appropriate enforcement measures shall be applied, in particular aiming to prevent re-occurrence in the case of documented illegal use (SANCO-E.2(04)D/521927).[19]

Nifursol was authorised in the EU as a feed additive for the first time by Commission Directive 82/822/EEC[20] and amended by Commission Directive 89/23/EEC[21] for the prevention of histomoniasis in turkeys. Three preparations were authorised with maximum nifursol contents of 14.6 %, 44 % and 50 %. The carriers for the three preparations were also regulated, which were maize starch and 12 %, 33 % or 34 % of soya bean oil, respectively. The Directive stipulated that the nifursol content in the complete feedingstuff should be between 50 mg/kg (minimum content) and 75 mg/kg (maximum content). The use of nifursol-containing feedingstuffs was prohibited at least 5 days before slaughter. Following the SCAN opinion on nifursol, the authorisation of nifursol as a feed additive was withdrawn with effect from 31 March 2003 by Council Regulation (EC) No 1756/2002.[22]

3. Methods of analysis

3.1. Sampling and storage

Most of the sampling of food, and of related materials, for nitrofurans testing in foods of animal origin is undertaken in the context of the national residue monitoring plans as specified in Council Directive 96/23/EC,[23] with residue testing undertaken in accordance with Commission Decision 2002/657/EC. For details of the protocols and procedures specified for such sampling and testing, see Section 5.2.1 of this opinion.

Commission Decision 2002/657/EC states that samples shall be obtained, handled and processed in such a way that there is a maximum chance of detecting the substance. Sample handling procedures shall prevent the possibility of accidental contamination or loss of analytes. To achieve this goal, samples are stored in suitable, secure, clearly identified containers and in conditions such as frozen storage (animal tissues, urine, blood plasma, milk, fish and shellfish, feed water) or at refrigerated/ambient temperatures (eggs, honey, animal feed) prior to analysis.

3.2. Determination of nitrofurans and their marker metabolites

Initially, testing for residues of nitrofurans in animal tissues was conducted using methods directed at the parent compounds, using high-performance liquid chromatography–ultraviolet (HPLC-UV) (Vroomen et al., 1986; Degroodt et al., 1992; Bellomonte et al., 1993) and later using liquid chromatography–mass spectrometry (LC-MS) (McCracken et al., 1995) techniques. However, it was unusual to find any residues of nitrofurans using these methods directed at the parent compounds. Studies on furazolidone showed that residues of the parent compound are highly unstable in treated animals (Nouws and Laurensen, 1990; McCracken et al., 1995), but that metabolites containing AOZ are covalently bound to tissue protein (Vroomen et al., 1986; Hoogenboom et al., 1991c) and that these metabolites persist for much longer than the parent compound (Hoogenboom et al., 1992a). Nitrofuran drugs contain a side-chain connected via an azomethine bond to the nitrofuran moiety. This bond is unstable under acidic conditions, a feature used by Buzard et al. (1956) for a generic method for nitrofuran drugs, starting with acid treatment and subsequent detection of the nitrofuran ring after derivatisation. By reversing this feature, it was shown that AOZ could be released from tissue-bound metabolites in pig liver under mildly acidic conditions followed by derivatisation with

[19] http://ec.europa.eu/food/fs/rc/scfcah/biological/rap16_en.pdf

[20] Forty-first Commission Directive of 19 November 1982 amending the annexes to Council Directive 70/524/EEC concerning additives in feedingstuffs. OJ L 347, 07.12.1982, p. 16–19.

[21] Commission Directive 89/23/EEC of 21 December 1988 amending the annexes of Council Directive 70/524/EEC concerning additives in feedingstuffs. OJ L 11, 14.1.1989, p. 34–35.

[22] Council Regulation (EC) No 1756/2002 of 23 September 2002 amending Directive 70/524/EEC concerning additives in feedingstuffs as regards withdrawal of the authorization of an additive and amending Commission Regulation (EC) No 2430/1999. OJ L 265, 03.10.2002, p. 1–2.

[23] Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC. OJ L 125, 23.5.1996, p. 10–32.

2-nitrobenzaldehyde (NBA) to form 3([(2-nitrophenyl) methylene]-amine)-2-oxazolidinone (NPAOZ), which was determined by HPLC-UV (Hoogenboom et al., 1991c). It was shown that this HPLC-UV-based method also worked for the detection of bound residues of other nitrofurans such as furaltadone, nitrofurantoin and nitrofurazone (Hoogenboom and Polman, 1993).

This principle became the basis for an analytical method for the determination of residues of tissue-bound metabolites of furazolidone and tissue-bound metabolites of furaltadone, such as 3([(2-nitrophenyl) methylene]-amine)-5-methylmorpholino-2-oxazolidinone (NPAMOZ), in pig liver, using both HPLC and LC-MS techniques (Horne et al., 1996). Subsequently, a method based on similar acidic hydrolysis and NBA derivatisation was developed for tissue-bound metabolites of four nitrofurans, using AOZ, AMOZ, AHD and SEM as the marker metabolites, in pig muscle, with determination of residues by liquid chromatography–tandem mass spectrometry (LC-MS/MS) (Leitner et al., 2001). The limits of quantitation (LOQs) for these methods were in the order of 5–10 µg/kg. The analytical methodology for tissue-bound nitrofuran metabolites was further developed through application of solid phase extraction (SPE) to improve clean-up of extracts and concentrate the derivatised marker metabolites (Conneely et al., 2003). An LC-MS/MS method, with hexane washing following hydrolysis and derivatisation of the marker metabolites and further clean-up by SPE on a reversed-phase polymeric sorbent, was applied to poultry and shrimp samples with an LOQ of 0.5–1.0 µg/kg (Edder et al., 2003).

Because tissue-bound metabolites of the nitrofurans are the principal target for residue analysis, testing for the parent compounds is limited, generally, to samples of animal feed and feed water, whereas samples of animal tissues, urine, blood plasma, milk, fish and shellfish, eggs and honey are tested for the nitrofuran marker metabolites.

3.2.1. Extraction and sample clean-up

For nitrofuran marker metabolites, a combined acid hydrolysis with hydrochloric acid and derivatisation with NBA is performed to release the protein-bound residues. Where determination of only the protein-bound metabolites is required, for example to specifically identify the SEM marker metabolite of nitrofurazone from other sources of SEM (see Section 3.3), a series of washing steps with organic solvents (methanol, ethanol, diethyl ether) may be performed prior to the hydrolysis/derivatisation step (Hoogenboom et al., 1991c). Following derivatisation, extraction of the nitrophenyl marker metabolites is undertaken using ethyl acetate (O'Keeffe et al., 2004).

Further clean-up of the sample extracts may be performed using SPE, particularly for honey samples (Tribalat et al., 2006; Lopez et al., 2007; O'Mahony et al., 2011), or washing with hexane (Bock et al., 2007). Some alternative approaches have been proposed for the release and derivatisation of marker metabolites, such as protease digestion of samples and extraction of the derivatised marker metabolites using mixed-mode cation exchange SPE instead of ethyl acetate (Cooper et al., 2007; Stastny et al., 2009), accelerated solvent extraction with methanol/5 % trichloroacetic acid (1/1, v/v) (Tao et al., 2012), incubation at 55 °C instead of at 37 °C (Verdon et al., 2007) or incubation in a microwave oven (Palaniyappan et al., 2013).

In the case of the parent nitrofurans, extraction from feed samples is carried out using solvent extraction with ethyl acetate, with acetonitrile or with a mixture of acetonitrile and /methanol (1/1, v/v). Typically, the solvent extract is subjected to further clean-up with SPE, using reversed-phase (C18 or polymeric sorbents), aminopropyl or neutral alumina sorbent chemistries. For water samples, parent nitrofurans are extracted using reversed-phase SPE with C18 or polymeric sorbents.

3.2.2. Screening methods

Screening methods should measure nitrofuran marker metabolites with sufficient sensitivity to satisfy regulatory requirements, currently at the MRPL of 1.0 µg/kg for poultry meat and aquaculture products (Annex II of Commission Decision 2002/657/EC). Screening methods for nitrofuran marker

metabolites include immunoassays (enzyme-linked immunosorbent assays (ELISA), lateral flow immunoassays, biosensors) and HPLC techniques.

Immunoassays have been very widely applied as screening methods for individual nitrofuran marker metabolites, typically directed at the nitrophenyl derivatives following acid hydrolysis and derivatisation with nitrobenzaldehyde, as described in Section 3.2.1 above.

An ELISA was developed for the determination of **NPAOZ** in prawns with a limit of detection (LOD) of 0.1 µg/kg and a detection capability (CCβ)[24] of < 0.7 µg/kg (Cooper et al., 2004). Franek et al. (2006) developed an ELISA for NPAOZ in eggs with a CCβ of 0.3 µg/kg. For fish, a number of NPAOZ ELISAs have been developed with LOD/LOQ values of 0.1/0.3 µg/kg (Cheng et al., 2009; Tsai et al., 2009; Liu et al., 2010b). For pork, chicken and beef muscle and liver samples, ELISA tests for NPAOZ were developed with a CCβ value of 0.4 µg/kg (Diblikova et al., 2005), with LOD values of 0.3–0.4 µg/kg (Chang et al., 2008) and with an LOD value of 1.0 µg/kg (Nesterenko et al., 2012).

A number of ELISA methods have been reported for the determination of **NPAMOZ** in shrimps and fish samples with reported LOD values of 0.1–0.3 µg/kg and LOQ or CCβ values of 0.3–0.36 µg/kg (Pimpitak et al., 2009; Shen et al., 2012; Sheu et al., 2012; Yang et al., 2012; Liu et al., 2013). Other ELISA methods were developed and applied to the determination of NPAMOZ in chicken, pork, fish and shrimp samples, reporting LOD values ranging from < 0.1 to 0.34 µg/kg (Luo et al., 2012; Xu et al., 2013; Shu et al., 2014). A number of ELISA methods have also been reported for direct determination of AMOZ, without derivatisation, with reported LOD values of 0.4 µg/kg (Song et al., 2012; Yan et al., 2012).

ELISA methods have been described for the determination of 1([(2-nitrophenyl) methylene]-amine)-hydantoin (**NPAHD**) in pork, fish, shrimps and chicken with LOD values ranging between 0.09 and 0.15 µg/kg (Wenxiao et al., 2012) and in shrimps with an LOD value of 0.11 µg/kg (Chadseesuwan et al., 2013).

ELISAs have been developed for [(2-nitrophenyl) methylene]-semicarbazide (**NPSEM**) in chicken (Cooper et al., 2007), pork (Vass et al., 2008a) and eggs (Vass et al., 2008b) with CCβ values of 0.25, 0.3 and 0.3 µg/kg, respectively.

Not published ELISA methods have been identified for the nitrophenyl derivative of the nifursol marker metabolite, 3[(2-nitrophenyl) methylene]-5-dinitrosalicylic acid hydrazide (**NPDNSH**).

The performance of commercial ELISA kits has been assessed by a number of authors and reported to be suitable for nitrofuran marker metabolite screening. Krongpong et al. (2008) reported that an ELISA kit was capable of detecting AOZ at 1.0 µg/kg in eel samples with excellent accuracy and precision. Dimitrieska-Stojkovic et al. (2012) validated test kits for AOZ, AMOZ, AHD and SEM in liver, eggs and honey and estimated CCβ values to be in the range of 0.56 to 0.68 µg/kg for all analytes. Shen et al. (2012) estimated the LODs for AOZ, AMOZ, AHD and SEM to be 0.02, 0.06, 0.13 and 0.04 µg/kg, respectively, for the application of commercial ELISA kits to the analysis of pork, chicken, fish and shrimp samples. Jester et al. (2014) tested commercial ELISA kits for AOZ and AMOZ in fish samples and reported that the LODs were 0.05 and 0.2 µg/kg, respectively.

Using an alternative carboxybenzaldehyde derivatisation, lateral flow immunoassays were developed for 1([(2-carboxyphenyl) methylene]-amine)-hydantoin (CPAHD) and [(2-carboxyphenyl) methylene]-semicarbazide (CPSEM) in pork with visual LODs of 1.4 and 0.72 µg/kg, respectively (Tang et al., 2011a, b). Li et al. (2013) developed a lateral flow immunoassay for underivatised AMOZ in chicken and pork samples and reported a visual LOD of 0.3 µg/kg.

[24] CCβ is the detection capability, meaning the smallest content of the substance that may be detected, identified and/or quantified in a sample with an error probability of β.

A number of biosensor assays for nitrofuran marker metabolites have been developed, including a chemiluminescence-based biochip array sensing technique for the nitrophenyl derivatives of AOZ, AMOZ, AHD and SEM in honey samples with CCβ values of < 0.5 µg/kg for AOZ, AMOZ and AHD and of < 0.9 µg/kg for SEM (O'Mahony et al., 2011).

Following the identification of tissue-bound metabolites of nitrofurans as the target analytes for residue analysis, HPLC-UV determination of the nitrophenyl derivatives was used initially (Hoogenboom et al., 1991c; Horne et al., 1996; Conneely et al., 2003). These methods had LOQ values of 2–10 µg/kg, which are not suitable for testing at the MRPL of 1 µg/kg. Recently, methods based on HPLC with diode-array detection (DAD) and fluorescence detection (FL) have been developed for nitrofuran marker metabolites, using 2-naphthaldehyde and 2-hydroxy-1-naphthaldehyde, respectively, as the derivatising reagent following acid hydrolysis. Following extraction with ethyl acetate, the derivatised nitrofuran marker metabolites were determined in shrimps by HPLC-DAD with LOQ values of 0.70–0.91 µg/kg (Chumanee et al., 2009) and in shrimp and pork muscle by HPLC-FL (λ_{ex} 395 nm; λ_{em} 463 nm) with LOQ values of 0.63–0.86 µg/kg (Sheng et al., 2013; Du et al., 2014).

The parent nitrofurans have been determined in feed samples using ELISA (Li et al., 2009, 2010), chemiluminescence (Thongsrisomboon et al., 2010; Liu et al., 2012a), HPLC-UV (McCracken et al., 1997; Wang and Zhang, 2006; Vinas et al., 2007) and LC-MS/MS (Barbosa et al., 2007b) techniques and in water samples using HPLC-UV (Pietruszka et al., 2007; Vinas et al., 2007) and LC-MS/MS (Ardoosngnearn et al., 2014) techniques. The reported LODs for such methods range from < 1 µg/kg to > 1 mg/kg; in the case of parent nitrofurans, the MRPL of 1 µg/kg for nitrofuran marker metabolites in foods of animal origin does not apply.

3.2.3. Confirmatory methods

LC-MS/MS has become the most widely used methodology for confirmatory analysis of nitrofuran marker metabolites in a broad range of sample types. Single quadrupole LC-MS has been shown not to be sufficiently sensitive (Cooper and Kennedy, 2005) or selective (Tribalat et al., 2006) for the determination of marker metabolites at the MRPL of 1 µg/kg. Most of the published LC-MS/MS methods are multi-residue methods, covering AOZ, AMOZ, AHD and SEM, with a few methods also including DNSH. Some other methods have been developed for only one or two marker metabolites. Typical MS conditions used for the confirmatory analysis of nitrofuran marker metabolites are a positive electrospray interface (ESI) with two precursor-to-product ion transitions being monitored for each marker metabolite (O'Keeffe et al., 2004). Sample treatment, prior to LC-MS/MS determination of the marker metabolites, typically involves acid hydrolysis and NBA derivatisation, with ethyl acetate or acetonitrile extraction and/or hexane washing and reversed-phase polymeric sorbent SPE clean-up.

LC-ESI-MS/MS methods for the determination of AOZ, AMOZ, AHD and SEM in **animal tissues** have been applied to poultry, pork, beef and rabbit muscle and liver samples. The range of values of the decision limit (CCα),[25] CCβ, LOD and LOQ for the various analytes by these methods are 0.11–0.45, 0.19–0.88, 0.01–0.2 and 0.5 µg/kg, respectively (Finzi et al., 2005; Mottier et al., 2005; Barbosa et al., 2007a; Xia et al., 2008; Ryad et al., 2013). A number of papers have been published on the determination of DNSH in turkey and chicken muscle and liver, with reported CCβ values of ≤ 0.1 µg/kg (Kaufmann et al., 2004; Mulder et al., 2005; Vahl, 2005; Zuidema et al., 2005). A method directed at the analysis of all five marker metabolites (AOZ, AMOZ, AHD, SEM, DNSH) in turkey muscle reported CCα values of 0.08–0.20 µg/kg and CCβ values of 0.10–0.25 µg/kg (Verdon et al., 2007).

Because of the high numbers of samples of **shrimps** (prawns) imported into the EU that have been found to contain residues of nitrofuran marker metabolites in the early years of this century (Kennedy

[25] CCα is the decision limit at and above which it can be concluded with an error probability of α that a sample is non-compliant.

et al., 2003), considerable attention has been given to developing methods for testing shrimps. Methods have been described using an atmospheric pressure chemical ionisation (APCI) interface with reported LOD/LOQ values in the range of 0.05–0.3/0.1–0.5 µg/kg (Chu and Lopez, 2005; An et al., 2012) or an ESI interface with reported CCα/CCβ values in the range of 0.08–0.36/0.12–0.61 µg/kg (Douny et al., 2013; Hossain et al., 2013). A further method that uses 2-naphthaldehyde, rather than 2-nitrobenzaldehyde, as the derivatising reagent reports LOD/LOQ values of 0.16–0.27/0.54–0.90 µg/kg (Chumanee et al., 2009).

For **fish**, methods are described with reported CCα values of 0.19 to 0.43 µg/kg and CCβ values of 0.23 to 0.54 µg/kg (Tsai et al., 2010) and LOD values of 0.03 to 0.15 µg/kg (Zhao et al., 2011). An alternative method involving accelerated solvent extraction was described by Tao et al. (2012); CCα values of 0.07 to 0.13 µg/kg and CCβ values of 0.31 to 0.49 µg/kg are reported.

Methods for AOZ, AMOZ, AHD and SEM in **egg** samples (Bock et al., 2007; Sniegocki et al., 2008) by LC-MS/MS using a positive ESI have reported CCα values of 0.03 to 0.22 µg/kg for the former method and CCα values of 0.16 to 0.25 µg/kg and CCβ values of 0.22 to 0.36 µg/kg reported for the latter method. In addition to these multi-nitrofuran methods for eggs, a number of methods have been published for individual marker metabolites in eggs. For SEM, methods with reported CCα values of 0.41 to 0.91 µg/kg and CCβ values of 0.46 to 0.97 µg/kg for eggs and egg products (Szilagy and de la Calle, 2006) and CCα/CCβ values of 0.20/0.25 µg/kg for eggs (Stastny et al., 2009) have been reported. Barbosa et al. (2012a) describe a method for AMOZ and DNSH in eggs with reported CCα/CCβ values of 0.1/0.5 µg/kg for AMOZ and 0.3/0.9 µg/kg for DNSH.

For **honey** samples, methods using LC-ESI-MS/MS with CCα values of 0.07 to 0.46 µg/kg and CCβ values of 0.12 to 0.56 µg/kg (Khong et al., 2004), LOD values of 0.2 to 0.6 µg/kg (Tribalat et al., 2006) and an LOQ value of 0.25 µg/kg (Lopez et al., 2007) are reported.

Methods for AOZ, AMOZ, AHD and SEM in **milk** samples (Chu and Lopez, 2007; Rodziewicz, 2008) use APCI or positive ESI. An LOD of < 0.2 µg/kg is reported for the former method and CCα values of 0.12 to 0.29 µg/kg and CCβ values of 0.15 to 0.37 µg/kg are reported for the latter method.

Determination of nitrofuran marker metabolites and parent compounds in pig and chicken **eyes** by LC-ESI-MS/MS have been described by Cooper and Kennedy (2005) and Cooper et al. (2008b). This analytical approach was adopted to take advantage of the higher concentration of drug residues in eye tissues and, particularly, to confirm the source of SEM as a nitrofuran marker metabolite through confirmation of the parent compound nitrofurazone in the eye. Another approach to unequivocal identification of nitrofurazone usage is the determination of the open-chain cyano-metabolite of nitrofurazone (Wang et al., 2010). Muscle samples from nitrofurazone-treated fish are analysed by LC-MS/MS; the cyano-metabolite is measurable for up to 14 days after treatment with nitrofurazone, compared with only 4 days for the parent compound. The authors suggest that the cyano-metabolite can be used as an alternative confirmatory marker for monitoring the use of nitrofurazone in fish. A method for the determination of marker metabolites in bovine, ovine, equine and porcine **plasma** has been developed as a method for pre-slaughter, on-farm testing for illicit use of nitrofuran drugs. The plasma samples are derivatised with NBA and analysed by ultra-(U)HPLC-MS/MS, with reported CCα values for AOZ, AHD, SEM and AMOZ of 0.059, 0.054, 0.070 and 0.071 µg/kg, respectively (Radovnikovic et al., 2011). An alternative method for pre-slaughter, on-farm testing for illicit use of nitrofuran drugs is based on **urine**, using SPE on a reversed-phase polymeric sorbent to extract the derivatised marker metabolites and analysis by LC-ESI-MS/MS. For AOZ, AHD, SEM and AMOZ in urine, the CCα/CCβ values were 0.11–0.34/0.13–0.43 µg/kg (Rodziewicz and Zawadzka, 2013).

3.3. SEM analysis

A particular problem with using SEM as an unequivocal marker for nitrofurazone arises owing to the occurrence of SEM in food from a number of other sources. Apart from its occurrence as a marker metabolite for nitrofurazone, SEM or compounds from which SEM may be released may occur in food

(1) as a migration or breakdown product from azodicarbonamide which has been used both as a blowing agent to foam the plastic sealing gaskets on metal lids of food jars and as a flour treatment agent in bread production (Becalski et al., 2004; Stadler et al., 2004); (2) as a reaction product formed between hypochlorite, used in cleaning, and carrageenan or powdered egg white (Hoenicke et al., 2004); and (3) as a naturally occurring compound from which SEM may be released in shrimps, prawns and crayfish and in honey (Saari and Peltonen, 2004; Van Poucke et al., 2011; McCracken et al., 2013; Crews, 2014). It should be noted that the use of azodicarbonamide has been prohibited within the EU for use both as a blowing agent (Commission Directive 2004/1/EC[26]) and as a food additive for flour, not being included in the Community list of food additives approved for use in foods (Annex II of Regulation (EC) No 1333/2008[27]), but it may continue to be used in other countries. Because no alternative marker residue for nitrofurazone has been identified to date, particular steps need to be taken when positive screening results for SEM are found (Sanders, 2003; Points et al., 2015). Some examples of these steps include the following: (1) testing for the marker metabolite for nitrofurazone in breaded food products should be carried out on only the animal tissue part of the product, (2) the inner core of products such as shrimps, prawns and crayfish should be tested for the marker metabolite for nitrofurazone, as the naturally occurring SEM occurs in only the outer part, and (3) the sample should be extensively washed with a range of organic solvents to remove any free SEM from the sample prior to the hydrolysis and derivatisation step for SEM as the marker metabolite for nitrofurazone.

3.4. Analytical quality assurance: performance criteria, reference materials and proficiency testing

The performance criteria for methods used to test for nitrofuran marker metabolites are those laid down in Commission Decision 2002/657/EC for screening and confirmatory methods to be used for Group A substances, i.e. substances having anabolic effects and unauthorised substances, such as nitrofurans, which are included in Table 2 of Commission Regulation (EU) No 37/2010. Methods must have a satisfactory level of performance for the characteristics of specificity, trueness, ruggedness and stability of the analyte in standard solutions and in test matrices. The methods must be validated for recovery, repeatability, within-laboratory reproducibility, calibration curves, CCα and CCβ in accordance with procedures specified in the Decision or equivalent procedures.

Isotopically labelled nitrofuran marker metabolites, such as D_4-AOZ, D_5-AMOZ, $^{13}C^{15}N_2$-SEM and $^{13}C_3$-AHD, are available commercially for use as internal standards. No certified reference materials for nitrofuran marker metabolites are commercially available to date. There is a report (PhD thesis) on the preparation of two certified reference materials of prawns containing AOZ at levels of 3.0–3.5 and 14–15 µg/kg (Muaksang, 2009).

Several proficiency tests and interlaboratory studies have been reported for nitrofuran marker metabolites in various food products. In 2003, the European Reference Laboratory prepared shrimp samples (three containing AOZ, two containing AOZ plus SEM and three blank samples) for distribution to 20 laboratories for analysis by LC-MS/MS methods. Four of the laboratories reported one or more false-negative or false-positive results and the rate of laboratories having satisfactory z-scores was 70 to 87 % for AOZ and 64 to 69 % for SEM; the assigned marker metabolite contents in the incurred samples were 0.9–1.2 µg/kg AOZ and 1.3–1.4 µg/kg SEM (Hurtaud-Pessel et al., 2006). An interlaboratory validation study was organised by the Institute for Reference Materials and Measurements (IRMM) to evaluate the effectiveness of an LC-MS/MS method for the determination of SEM in whole egg and egg powder samples. Five samples each of whole egg and of egg powder were analysed by 12 laboratories; the relative standard deviations for repeatability and for reproducibility ranged from 2.9 to 9.3 % and from 22.5 to 38.1 %, respectively, demonstrating that the method showed acceptable within- and between-laboratory precision (De la Calle and Szilagyi, 2006).

[26] Commission Directive 2004/1/EC of 6 January 2004 amending Directive 2002/72/EC as regards the suspension of the use of azodicarbonamide as blowing agent. OJ L 7, 13.1.2004, p. 45–46.

[27] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.

In the UK, the Food Analysis Performance Assessment Scheme (FAPAS) provides samples of honey and prawns containing nitrofuran marker metabolites for testing.[28]

3.5. Concluding comments

With some exceptions, both screening and confirmatory methods for nitrofuran marker metabolites in foods of animal origin use acid hydrolysis and NBA derivatisation of the released marker metabolites. Screening methods for the resulting nitrophenyl derivatives is generally undertaken by ELISA or biosensor methods, providing sufficient analytical sensitivity to meet the MRPL of 1 µg/kg. Confirmatory methods are based on LC-MS/MS and this technology typically provides CCα values of < 0.3 µg/kg and CCβ values of < 0.5 µg/kg for the range of sample types, again adequately meeting the MRPL of 1 µg/kg.

4. Assessment of the appropriateness of using marker metabolites of nitrofurans for the reference point for action for foods of animal origin

Nitrofuran parent compounds have a short *in vivo* half-life due to extensive metabolism, primarily a reduction of the nitro-group, such that they do not occur generally as residues in foods of animal origin (Nouws and Laurensen, 1990; McCracken et al., 1995). Therefore, monitoring of nitrofuran residues in livestock based on the identification of the parent compounds is not appropriate. Instead, an approach based on marker metabolites has been adopted for monitoring purposes, with the particular marker metabolites chosen to reflect residues of nitrofurans which persist in treated animals. The nitroreduction results in the formation of reactive metabolites able to bind covalently to tissue macromolecules, including proteins, which, in food-producing animals, have relatively long half-lives, persisting for several weeks in edible tissues (Vroomen et al., 1986; Hoogenboom et al., 1991c, 1992a; Vass et al., 2008c). Side-chains may be released from these protein-bound metabolites, namely AOZ, AMOZ, AHD, SEM and DNSH in the case of furazolidone, furaltadone, nitrofurantoin, nitrofurazone and nifursol, respectively. These side-chains are therefore excellent marker metabolites for the presence of protein-bound residues, following their release by sample treatment with acid and derivatisation with nitrobenzaldehyde (Hoogenboom et al., 1991c; Hoogenboom and Polman, 1993; Horne et al., 1996; Leitner et al., 2001).

As nitrofuran parent compounds do not persist as residues in animal tissues and do not occur at concentrations comparable to those of the marker metabolites (as protein-bound adducts), the marker metabolites AOZ, AMOZ, AHD, SEM and DNSH are appropriate for identifying the illicit use of nitrofurans. Other nitrofuran metabolites that persist and that are at concentrations higher than the marker metabolites AOZ, AMOZ, AHD, SEM and DNSH have not been identified. Therefore, these marker metabolites are appropriate for the RPA for foods of animal origin.

In the case of SEM, there is a problem with its use as a marker metabolite for nitrofurazone in that SEM may occur as a residue in some foods from other sources (Becalski et al., 2004; Hoenicke et al., 2004; Saari and Peltonen, 2004; Stadler et al., 2004; Van Poucke et al., 2011; McCracken et al., 2013; Crews, 2014), potentially giving rise to false-positive results for illicit use of nitrofurazone. However, a suitable alternative marker metabolite for nitrofurazone has not been identified. Wang et al. (2010) suggested the determination of the open-chain cyano-metabolite of nitrofurazone in fish muscle as an alternative marker metabolite, but this has not been applied more widely; in pigs, it was shown that the cyano-metabolite has a much shorter half-life than the protein-bound residues (Vroomen et al., 1987a). Instead, as SEM is an appropriate marker metabolite for nitrofurazone, the problem of potential false-positive results for illicit use of nitrofurazone has been addressed by appropriate analytical strategies (Sanders, 2003; Points et al., 2015) (see Section 3.3).

[28] http://fapas.com/proficiency-testing-schemes/fapas/

5. Occurrence of nitrofurans in food

5.1. Previously reported occurrence results

Owing to the previous use of nitrofurans as veterinary drugs or feed additives, most controls on the presence of nitrofuran marker metabolites are focused on foods of animal origin. However, SEM can also be present in food from sources other than nitrofurazone use (see Appendix A). Because the MRPL for nitrofuran marker metabolites was established by Commission Decision 2002/657/EC (see Section 2), the EFSA Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) considered occurrence data for samples that have been collected since 2002. It should be noted that some of the studies described in this section may also be included in the databases described in Section 5.2, relating to samples taken in national residue monitoring plans. The information presented below provides examples of the occurrence of nitrofuran marker metabolites in foods of animal origin.

5.1.1. Meat and meat products

In 2002, the Food Standards Agency (FSA) analysed AOZ, AMOZ, AHD and SEM in chicken meat (n = 45). The reporting limit was 0.3 µg/kg for AOZ, AMOZ and AHD, and was 1 µg/kg for SEM. AMOZ was detected in four samples with concentrations ranging from 0.55 to 18.19 µg/kg and AOZ was detected in one sample with a concentration of 0.63 µg/kg. None of the samples contained more than one nitrofuran marker metabolite and AHD and SEM were not detected (FSA, 2002).

Meat samples (n = 226) from various species such as broilers, turkeys, quails, rabbits, bovine and swine were analysed for the presence of AOZ, AMOZ, AHD and SEM. Samples had been collected in Portugal in 2002 under the Portuguese residue monitoring plan and analysis was done by LC-MS/MS (CCα/CCβ: 0.29/0.34, 0.20/0.32, 0.45/0.88 and 0.15/0.46 µg/kg, respectively). From the 226 samples, 78 contained AMOZ at a concentration above the MRPL of 1 µg/kg. Most non-compliant samples were broiler meat (n = 61), but non-compliant samples were also reported for turkeys (n = 11), quails (n = 5) and pigs (n = 1). The average concentration of AMOZ in non-compliant samples was 6.3 µg/kg for broilers, 125 µg/kg for turkeys and 5.8 µg/kg for quails. No results were reported for the other nitrofuran marker metabolites tested (Barbosa et al., 2007a).

Poultry and rabbit samples (n = 55 and n = 8, respectively) taken from the Swiss market and mainly originating from Asian countries (year of sampling not indicated) were analysed for AOZ, AMOZ, AHD and SEM using LC/MS-MS (LOD/LOQ: 0.2/0.5 µg/kg for AOZ, AMOZ and SEM, and 2.0/5.0 µg/kg for AHD). AOZ was found in 20 poultry samples (36 %, range: 0.6–895 µg/kg) and one rabbit sample (13 %, concentration: 5.1 µg/kg). The other nitrofuran marker metabolites were not detected (Edder et al., 2003).

A survey on pork (n = 1 500) was undertaken across 15 European countries for AOZ, AMOZ, AHD and SEM. Sampling was done at retail and in pig slaughterhouses in 2002. Analysis was done by LC-MS/MS and LOQs were 0.1µg/kg for AOZ and AMOZ, 0.5 µg/kg for AHD and 0.2 µg/kg for SEM. In 12 samples (0.8 %), measurable nitrofuran marker metabolites were detected. AOZ was quantified in one sample from Portugal and one sample from Greece (range: 0.3–3.0 µg/kg). AMOZ was quantified in one sample from Italy and nine samples from Portugal (range: 0.2–1.0 µg/kg). No measurable concentrations of AHD and SEM were shown and none of the samples contained more than one nitrofuran marker metabolite (O'Keeffe et al., 2004).

In Denmark, chicken and turkey meat was sampled in retail outlets in 2003 and analysed for the presence of DNSH by LC-MS/MS (CCα/CCβ: 0.05/0.08 µg/kg). DNSH was not detected in any of the chicken meat samples (n = 16), but 10 of the 37 samples of turkey meat contained DNSH (range: 0.05–0.6 µg/kg) (Vahl, 2005).

Meat-based products (chicken and pork) mainly of Asian origin were tested for the presence of AOZ, AMOZ, AHD and SEM. Analysis was done by LC/MS-MS (CCα/CCβ: 0.11/0.19, 0.12/0.21, 0.21/0.36 and 0.20/0.34 µg/kg, respectively). More than 100 samples were tested (precise number and

year of sampling not indicated). AOZ was detected in 15 % of the samples (median: 0.6 µg/kg; maximum: 193 µg/kg) and AMOZ was detected in 10 % of the samples (median: 0.5 µg/kg; maximum: 9 µg/kg). SEM was most frequently detected (21 %, median: 10.9 µg/kg; maximum: 19.6 µg/kg), while AHD was not found in any of the samples (Mottier et al., 2005).

AOZ was analysed in samples of chicken liver (n = 90) collected from local supermarkets and retail stores in Turkey between December 2008 and August 2009. A commercial ELISA kit was used with an LOD of 0.1 µg/kg. AOZ was detected in 11 samples (12 %) with a concentration between 0.1 and 1 µg/kg (Yibar et al., 2012).

Radovnikovic et al. (2013) reported the analysis of samples of bovine liver (n = 316), ovine liver (n = 62), porcine liver (n = 104) and poultry liver (n = 80) that had been undertaken in the framework of the Irish national residue monitoring plan between 2009 and 2010. Analysis was done using UHPLC-MS/MS for AOZ, AMOZ, AHD and SEM (CCα: 0.067, 0.073, 0.074 and 0.064 µg/kg, respectively). SEM was detected in four ovine liver samples at concentrations ranging from 0.122 to 0.258 µg/kg. The other nitrofuran marker metabolites were not detected.

5.1.2. Honey

An LC-MS/MS method was used to analyse AOZ, AMOZ, AHD and SEM in more than 120 honey samples of different geographical origins that were collected from various honey suppliers and retail outlets in Switzerland in 2002 and 2003. The CCα/CCβs were 0.12/0.18, 0.07/0.12, 0.46/0.56 and 0.36/0.43 µg/kg, respectively. AMOZ and AHD were not detected in any of the samples. AOZ and SEM were detected in 14 and 21 % of the samples, with maximum concentrations of 5.1 and 24.5 µg/kg, respectively (Khong et al., 2004).

Between 2007 and 2009, 55 honey samples were collected from local apiaries in Romania and analysed for AOZ and AMOZ with a commercial ELISA kit (LOD/LOQ/CCα/CCβ not reported). AOZ was detected in six samples at concentrations ranging from 0.63 to 0.89 µg/kg and AMOZ was detected in five samples at concentrations ranging from 0.84 to 0.89 µg/kg (Simion et al., 2012).

Radovnikovic et al. (2013) reported the analysis of honey samples (n = 271) that had been undertaken in the framework of the Irish national residue monitoring plan and during an additional retail survey between 2009 and 2010. Analysis was done using UHPLC-MS/MS for AOZ, AMOZ, AHD and SEM (CCα: 0.093, 0.096, 0.138 and 0.090 µg/kg, respectively). SEM was detected in nine samples at concentrations ranging from 0.091 to 1.27 µg/kg. The other nitrofuran marker metabolites were not detected.

5.1.3. Fish and other seafood

Because SEM may be released from a naturally occurring compound in the shell of shrimps, prawns and crayfish (Saari and Peltonen, 2004; Van Poucke et al., 2011; McCracken et al., 2013; Crews, 2014), the part (e.g. meat, shell) of these shellfish tested in the studies below is described, where known.

Fish (n = 16) and shrimp samples (n = 157, details on part tested not given) taken from the Swiss market and mainly originating from Asian countries (year of sampling not indicated) were analysed for AOZ, AMOZ, AHD and SEM using LC/MS-MS (LOD/LOQ: 0.2/0.5 µg/kg for AOZ, AMOZ and SEM, and 0.5/1.0 µg/kg for AHD). Nitrofuran marker metabolites were found in 54 shrimp samples (34 %) and five fish samples (31 %). In shrimps, both AOZ (range: 0.5–324 µg/kg) and SEM (range: 0.7–227 µg/kg) were found, while, in fish samples, only AOZ (range: 0.9–68 µg/kg) was detected. The other nitrofuran marker metabolites were not detected (Edder et al., 2003).

Saari and Peltonen (2004) analysed SEM in crayfish (meat part tested) caught from rivers not near aquaculture farming that had been boiled in fresh salty water (year of sampling not indicated). The analysis was done by LC-MS/MS (LOD/LOQ: 0.04/0.4 µg/kg). SEM was quantified in all samples

(mean: 4.2 µg/kg; range: 0.7–12 µg/kg; n = 18). Tissue-bound SEM was detected in 12 samples and quantified in four samples (range: 0.4–0.6 µg/kg).

FSANZ reported the analysis of AOZ, AMOZ, AHD and SEM in prawn samples (n = 136, details on part tested not given). The limit of reporting for all nitrofuran marker metabolites was 1 µg/kg. AHD was not detected in any of the samples. AMOZ was detected in one sample at a concentration of 2.2 µg/kg and SEM was detected in a sample of dried prawn at a concentration of 8.9 µg/kg. AOZ was found in 10 samples at concentrations ranging from 1.1 to 40 µg/kg (FSANZ, 2004).

In the framework of a Canadian total diet study, 12 composite samples of marine, freshwater and canned fish and shrimps (meat part tested) were collected between 2002 and 2004. AOZ, AMOZ, AHD and SEM were analysed using LC-MS (LOD: 0.1 µg/kg for AOZ, AMOZ and AHD, and 0.4 µg/kg for SEM). AOZ was detected in two composite shrimp samples (1.3 and 0.5 µg/kg) and SEM was detected in one composite shrimp sample (0.8 µg/kg). The other nitrofuran marker metabolites were not detected (Tittlemier et al., 2007).

In Belgium, an increase of positive SEM analyses of prawns (*Macrobrachium rosenbergii*) was observed in 2008–2009 compared with other EU Member States. It was noted that Belgium analysed the whole prawns (meat and shell) while other countries used only the edible part (meat). Therefore, Van Poucke et al. (2011) analysed 12 samples of crustaceans for the occurrence of tissue-bound SEM in the meat and shell by LC-MS/MS (LOD: 0.5 µg/kg). SEM was detected in the shell of 11 samples at concentrations ranging from 1.5 to 12.6 µg/kg, while it was detected in the meat of only one sample at a much lower concentration (0.6 µg/kg) (Van Poucke et al., 2011).

Radovnikovic et al. (2013) reported the analysis of samples of prawns (n = 88, details on part tested not given), sea bass (n = 7), trout (n = 24) and salmon (n = 71) that had been undertaken in the framework of the Irish national residue monitoring plan and during an additional retail survey between 2009 and 2010. Analysis was done using UHPLC-MS/MS for AOZ, AMOZ, AHD and SEM (CCα: 0.041, 0.061, 0.057 and 0.064 µg/kg, respectively). SEM was detected in three prawn samples (reported range: 0.159–0.206 µg/kg) and one salmon sample (0.088 µg/kg). AOZ was detected in two prawn samples at a concentration above the MRPL (reported concentrations: 1.144 µg/kg and 1.626 µg/kg). AMOZ and AHD were not detected.

McCracken and co-workers analysed SEM using LC-MS/MS (CCα: 0.06 µg/kg) in wild-caught shrimps from 29 sites across Bangladesh (upstream, downstream or around *M. rosenbergii* aquaculture sites; year of sampling not indicated). Tissue-bound SEM was detected in approximately 65 % of the meat samples at concentrations below the MRPL of 1 µg/kg and concentrations were unrelated to sampling location, suggesting natural occurrence. In addition, higher concentrations were observed in the shell than in the meat, and higher concentrations were also observed in the outer meat layer (epidermis) than in the core meat (McCracken et al., 2013).

Crustacean samples (n = 17, details on part tested not given) were collected from local markets in Almeria (Spain; year of sampling not indicated) and analysed using UHPLC-MS/MS for AOZ, AMOZ, AHD and SEM (CCα/CCβ/LOD/LOQ: 1.5/1.6/0.5/1.0, 2.0/2.3/0.6/1.0, 2.0/2.2/0.8/1.0 and 2.6/3.1/0.6/1.0 µg/kg, respectively). The tested nitrofuran marker metabolites were not detected in any of the samples (Valera-Tarifa et al., 2013).

5.1.4. Eggs

Radovnikovic et al. (2013) reported the analysis of egg samples (n = 52) that had been undertaken in the framework of the Irish national residue monitoring plan between 2009 and 2010. Analysis was done using UHPLC-MS/MS for AOZ, AMOZ, AHD and SEM (CCα: 0.066, 0.079, 0.079 and 0.074 µg/kg, respectively). None of the marker metabolites was identified in the samples.

5.2. Current occurrence results

5.2.1. Data sources

Data on the occurrence of nitrofurans and their marker metabolites in food are not currently collected by EFSA. The only analytical results on nitrofurans and their marker metabolites present in the EFSA Chemical Occurrence database have been voluntarily submitted by the Czech Republic (584), Denmark (10 232) and Spain (40), and are all left-censored (LOD/CCα/CCβ: ≤ 1 µg/kg). Information on the above-mentioned data is presented in Appendix B, Table B.1. The Czech Republic and Denmark confirmed that the same data were also submitted to the EC database on residues of veterinary medicines, relating to the national residue monitoring plan (see below).

5.2.1.1. National residue monitoring plans

Council Directive 96/23/EC on measures to monitor certain substances and residues thereof in live animals and animal products requires that Member States draft a national residue monitoring plan for the groups of substances detailed in Annex I of this Directive. These plans must comply with the sampling rules in Annex IV of the Directive. Nitrofurans and their marker metabolites are in Group A6 of prohibited substances, as listed in Table 2 of Commission Regulation (EU) No 37/2010, for which MRLs cannot be established. These substances are not allowed to be administered to food-producing animals.

The minimum number of each species of animal to be controlled each year for all kinds of residues and substances is specified as a proportion of the animals of each species slaughtered in the previous year. In the case of Group A substances, substances having anabolic effects and unauthorised substances, a proportion of the total samples taken are to come from live animals or related materials (feed, drinking water, urine, faeces, etc.) on farms and the remainder of the samples are to be taken at the slaughterhouse. Each subgroup of Group A, such as Group A6, which includes nitrofurans and their marker metabolites, must be checked each year using a minimum of 5 % of the total number of samples to be collected for Group A. Sampling under the national residue monitoring plan should be targeted; samples should be taken on-farm and at slaughterhouse level with the aim of detecting illegal treatment.

Member States submit data on the occurrence of non-compliant results determined in the residue monitoring, including for nitrofurans and their marker metabolites, to the EC database on residues of veterinary medicines. Data on the occurrence of nitrofurans and their marker metabolites in food have been extracted from the EC database on residues of veterinary medicines. This database contains the annual sampling plan and the results from 2004 onwards[29] provided by all Member States. The results are reported as aggregate data with the following level of detail:

- animal category and animal products: bovines, pigs, sheep and goats, horses, poultry, aquaculture, milk, eggs, rabbits, farmed game, wild game and honey;
- production volume;
- sampling strategy: targeted, suspect, import and others;
- number of samples analysed for each substance group as defined in Annex I of Council Directive 96/23/EC and for each animal category or animal product;
- number of non-compliant results within each substance group or subgroup and within each animal category or animal product;
- place of sampling: farm or slaughterhouse.

[29] The results for 2013 currently present in the European Commission's database are provisional and will be complete and available at the end of 2014.

However, there is no indication of the sample matrix tested (muscle, blood, urine, kidney, fat, etc.) and no concentration for the chemical residue or contaminant detected in the sample is provided. In addition, the number of samples analysed for the individual substances are reported by the Member States only if there is at least one non-compliant sample for the substance in question. Where all samples are compliant, the number of samples analysed is not reported. Furthermore, where controls are carried out at farm and slaughterhouse, the total number of samples recorded may refer to samples taken at either farm or slaughterhouse, depending on where the non-compliant samples were found, and this may be on a substance group basis rather than on the individual substance basis. Where non-compliant samples were found at both farm and slaughterhouse, the number of samples represents the sum of samples taken at both sampling points.

Data on nitrofurans and their marker metabolites reported by Member States during 2002 and 2003 have been extracted from the Commission staff working papers on the implementation of national residue monitoring plans in the Member States in 2002 and 2003. Unfortunately, the data presented in these papers are not consistent with the reports for the following years. The number of samples analysed for each food category represents in most cases the total of samples for all prohibited substances. Only for the food categories of bovines, pigs, poultry, and sheep and goats does the number of samples represent those analysed for the Group A6 substances only, which includes nitrofurans and their marker metabolites.

5.2.1.2. Rapid Alert System for Food and Feed

The CONTAM Panel considered the Rapid Alert System for Food and Feed (RASFF) [30] database as another source of information on the occurrence of nitrofurans and their marker metabolites in food.

RASFF notifications mostly concern controls at the outer European Economic Area (EEA) borders at points of entry or border inspection posts when a consignment is not accepted for import into the EU. The second largest category of notifications concerns official controls on the internal market. A small number of notifications are triggered by an official control in a non-member country, where a risk found during its official controls concerning a product that may be on the market in one of the member countries is transmitted to the RASFF network.

After an inspection is conducted within a country and unfavourable results of the analysis are obtained, the risk needs to be evaluated, as does the probability that the product may be present on the market of other member countries. Notifications are provided when non-compliant samples for a contaminant are found, providing also quantified values. However, information on the total number of samples analysed, the number of compliant samples, the concentrations and the type of analysis undertaken is rarely provided.

Searches in the RASFF database were performed for the hazard category ‘veterinary residues’— nitrofuran (metabolite)— that had been notified between 01/01/2002 and 31/12/2014.

5.2.2. Distribution of samples across food categories

5.2.2.1. National residue monitoring plans

In the period 2002–2013, 842 294 targeted samples (ranging from about 58 000 to 91 000 per year) were analysed for Group A6 prohibited substances by the European Member States.[31] While this number includes all A6 prohibited substances, the number of samples analysed for nitrofuran marker metabolites is unknown. For nitrofurans and their marker metabolites, the results shown in the residue database are as follows:

[30] http://ec.europa.eu/food/safety/rasff/docs/rasff_annual_report_2013.pdf

[31] The data for 2013 were extracted from the database between January 2015 and February 2015 and are reflective of the database during this time period.

- There were 214 targeted samples reported to be non-compliant for nitrofurans and their marker metabolites distributed across the years, as shown in Table 1. Most cases were detected in 2002 in poultry. In subsequent years, there was no clear trend.
- The animal species in which nitrofurans and their marker metabolites were most commonly reported were poultry, bovines, and sheep and goats with 105, 35 and 23 non-compliant cases, respectively. Other categories for which non-compliant samples were reported include farmed game, pigs, honey, rabbits, aquaculture, horses and wild game (Table 1).
- The type of nitrofurans and their marker metabolites of which samples were found to be non-compliant are shown in Table 2, with the highest number of non-compliant cases for AMOZ owing to problems in poultry in 2002. AHD was detected in only a few cases.

Table 1: Number of non-compliant samples for nitrofurans and their marker metabolites (targeted sampling), by category, for the period 2002–2013

Category/year	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	Total per category
Bovines		1	2	3		3	2	5	4	7	6	2	35
Poultry	72	1	7	6	3	1	4	3	3	1	2	2	105
Aquaculture	1	1		1	1				1				5
Sheep/goats		1	7	3			1	4	1	4	1	1	23
Rabbits		1	1	2			1					1	6
Pigs	2	2	1	5				2		1	1		14
Horses				1									1
Farmed game	5			2			2	1		1	1		12
Wild game						1							1
Honey					1				10	1			12
Total per year	**80**	**7**	**18**	**23**	**5**	**5**	**10**	**15**	**19**	**15**	**11**	**6**	**214**

Table 2: Number of non-compliant samples, by nitrofuran and marker metabolite, for the period 2002–2013

	Furazolidone[(a)] /AOZ	Furaltadone[(a)]/ AMOZ	Nitrofurantoin[(a)]/ AHD	Nitrofurazone[(a)]/ SEM	Nitrofuran not specified[(b)]
2002		77			3
2003	1		2	1	3
2004	7	1		10	
2005	9	1	9	4	
2006	2	1		1	1
2007	1	2	2		
2008	2	6		2	
2009	2	1		12	
2010	13	2		4	
2011	1	4		10	
2012	2	1		8	
2013		3		3	
Total	**40**	**99**	**13**	**55**	**7**

AHD: 1-aminohydantoin; AMOZ: 3-amino-5-methylmorpholino-2-oxazolidinone; AOZ: 3-amino-2-oxazolidinone; SEM: semicarbazide.

(a): The CONTAM Panel noted the different ways of reporting among Member States, as some report the parent compound and others report the marker metabolite.

(b): The samples are reported as being analysed for nitrofurans without specifying the identity of the nitrofuran or the marker metabolite.

5.2.2.2. Rapid Alert System for Food and Feed

The findings in the RASFF database for nitrofurans and their marker metabolites for the period 2002–2014 are shown below:

- There were 808 notification events[32] reported for nitrofuran marker metabolites in food products (Table 3).
- The notifications covered the following product categories: cephalopods and products thereof, crustaceans and products thereof, eggs and egg products, farmed crustaceans and products thereof,[33] farmed fish and products thereof (other than crustaceans and molluscs),[33] fish and fish products, food additives and flavourings, honey and royal jelly, meat and meat products (other than poultry), poultry meat and poultry meat products, prepared dishes and snacks, wild-caught crustaceans and products thereof[33] and wild-caught fish and products thereof (other than crustaceans and molluscs).[33]
- The two products categories for which the highest numbers of notification events were reported were crustaceans and products thereof and poultry meat and poultry meat products, with 416 and 150 notification events, respectively. The majority of the cases were in 2002 and 2003, but in subsequent years there was no real trend.
- The notification events[34] were reported for the four nitrofuran marker metabolites (AOZ, AMOZ, SEM and AHD) across the years as seen in Table 4. Most reports were for AOZ and SEM, followed by AMOZ (incident in 2002), and there were only a few cases for AHD.

[32] The total number of notification events is not the sum of the total number of notifications, because one notification event may include more than one notification. Notification events include alerts, border rejections, information, information for attention, information for follow-up and news.

[33] This product category is no longer used in the RASSF database.

[34] One notification event could report more than one marker metabolite. There were 27 notification events where the marker metabolite was not specified (18 for 2002, one for 2003, two for 2008, one for 2009, one for 2010, two for 2011, one for 2012 and one for 2013).

Table 3: Number of notification events in the Rapid Alert System for Food and Feed database for nitrofurans and their marker metabolites in food, by category, for the period 2002–2014

Category	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
Cephalopods and products thereof										1	1		
Crustaceans and products thereof	68	11	31	33	54	33	50	87	11	10	9	5	14
Eggs and egg products		13											
Farmed crustaceans and products thereof	18	37											
Farmed fish and products thereof (other than crustaceans and molluscs)	1	28											
Fish and fish products	7	1	3	3	2	2	2	1		5			20
Food additives and flavourings		2											
Honey and royal jelly		2	5	4	1	1	1	3			2	1	
Meat and meat products (other than poultry)	7	12	3	2	1	7	1	3	1	4	1	4	4
Poultry meat and poultry meat products	88	55	4	2				1					
Prepared dishes and snacks	1												
Wild-caught crustaceans and products thereof	2	21											
Wild-caught fish and products thereof (other than crustaceans and molluscs)		1											
Total	**192**	**183**	**46**	**44**	**58**	**43**	**54**	**95**	**12**	**20**	**13**	**10**	**38**

Table 4: Number of notification events in the Rapid Alert System for Food and Feed database, by nitrofuran and marker metabolite in food, for the period 2002–2014

	Furazolidone/ AOZ	Furaltadone/ AMOZ	Nitrofurantoin/ AHD	Nitrofurazone/ SEM	Nitrofuran not specified[(a)]
2002	78	68	5	45	18
2003	95	27	2	69	1
2004	24	1		24	
2005	15	3		27	
2006	15	3		41	
2007	27	2	1	16	
2008	19			36	2
2009	12	1		83	1
2010	2			10	1
2011	9			10	2
2012	7			5	1
2013	4			5	1
2014	17			21	
Total	**324**	**105**	**8**	**392**	**27**

AHD: 1-aminohydantoin; AMOZ: 3-amino-5-methylmorpholino-2-oxazolidinone; AOZ: 3-amino-2-oxazolidinone; SEM: semicarbazide.
(a): The notification events do not specify the identity of the nitrofuran or the marker metabolite.

5.3. Food processing

Cooper and Kennedy (2007) studied the stability of nitrofuran marker metabolites during cooking and freezing. Analysis was done using LC-MS/MS (CCα/CCβ: < 1.0 µg/kg). Muscle and liver of pigs, treated with furazolidone, furaltadone, nitrofurantoin or nitrofurazone, were cooked by frying, grilling, roasting and microwaving. The concentrations of AOZ, AMOZ, AHD and SEM in uncooked liver were 51.3, 32.8, 41.0 and 40.2 µg/kg, respectively. Following correction for water loss during cooking, frying caused a reduction of the marker metabolite concentration in the liver of 6 to 21 % and roasting caused a reduction of 22 to 33 %. The concentrations of AOZ, AMOZ, AHD and SEM in uncooked muscle were 45.6, 72.3, 25.4 and 240.9 µg/kg, respectively. Following correction for water loss during cooking, grilling caused a reduction of the marker metabolite concentration in the liver of 0 to 14 % and similar results were observed for microwaving and roasting (0 to 15 %). Storage of liver and muscle at –20 °C for 8 months did not cause a significant reduction of the concentrations of the four studied marker metabolites.

Eggs from laying hens treated with nitrofurazone were used to study the effect of pasteurisation and spray drying on the concentration of nitrofurazone and SEM in liquid egg. The nitrofurazone concentration in whole egg decreased during processing from 502.1 to 177.6 µg/kg dry weight (d.w.) (65 % reduction), in yolk from 267.6 to 151.9 µg/kg d.w. (43 % reduction) and in albumen from 1 085.6 to 44.5 µg/kg d.w. (96 % reduction). The SEM concentration in whole egg decreased during processing from 676.5 to 330.7 µg/kg d.w. (51 % reduction), in yolk from 608.7 to 507.6 µg/kg d.w. (17 % reduction) and in albumen from 954.3 to 264.5 µg/kg d.w. (72 % reduction). The authors indicated that direct comparisons between residue concentrations in whole egg, yolk and albumen cannot be made, because the pooled samples were derived from different populations of eggs (Cooper et al., 2008a).

The effect of boiling on AOZ concentration in nitrofuran-positive eggs was studied by homogenising 13 eggs individually and splitting each egg into two parts. One part was immersed in water at 100 °C for 10 minutes and the other part was analysed without heat treatment. The AOZ concentration in the uncooked eggs was 0.76–0.97 µg/kg and was 2.04–2.55 µg/kg after cooking. No correction for water loss during cooking was described. The authors postulated that the observed increase ($p < 0.001$) may

be a result of enhanced efficiency of extraction in boiled samples and proposed that boiled eggs be used for the analysis of AOZ to obtain more reliable and more predictive results (Yibar et al., 2013).

Honey spiked with AOZ, AMOZ, AHD and SEM was processed to study the influence of the different processing steps (preheating, filtration, concentration and pasteurisation) on the concentration of these nitrofuran marker metabolites. The initial concentrations of the nitrofuran marker metabolites were 2.50, 1.83, 2.14 and 2.16 µg/kg, respectively. Overall, the initial concentration was reduced during processing by 56.6, 71.3, 90.4 and 88.4 %, respectively. For AMOZ, AHD and SEM, the highest reduction occurred during preheating (45 °C for 1 hour), namely 33.0, 74.9 and 76.9 %, while for AOZ the highest reduction (33.7 %) occurred during filtration. The authors attributed the losses of the marker metabolites from honey during processing to heat instability during preheating, lipophilicity and wax removal during filtration, and volatility during concentration heating (Jia et al., 2014).

Overall, only limited information about the effect of food processing on nitrofuran marker metabolites is available.

6. Food consumption

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database) was built in 2010 from existing detailed national information on food consumption provided by EU Member States and food consumption data for children obtained through an EFSA Article 36 project (Huybrechts et al., 2011). The Comprehensive Database contains results from a total of 32 different dietary surveys carried out in 22 different Member States covering more than 67 000 individuals (EFSA, 2011b).

Within the dietary studies, subjects are classified in different age classes as follows:

Infants:	< 12 months old
Toddlers:	≥ 12 months to < 36 months old
Other children:	≥ 36 months to < 10 years old
Adolescents:	≥ 10 years to < 18 years old
Adults:	≥ 18 years to < 65 years old
Elderly:	≥ 65 years to < 75 years old
Very elderly:	≥ 75 years old

Overall, the food consumption data gathered by EFSA in the Comprehensive Database are the most complete and detailed data currently available in the EU. However, it should be pointed out that different methodologies were used between surveys to collect the data and thus direct country-to-country comparisons can be misleading.

The CONTAM Panel considered that only chronic exposure to nitrofurans and their marker metabolites needed to be assessed. As suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011b), dietary surveys with only one day per subject were not considered, as they are not adequate to assess repeated exposure. Similarly, subjects who participated only one day in the dietary studies, when the protocol prescribed more reporting days per individual, were also excluded for the chronic exposure assessment. Thus, for chronic exposure assessment, food consumption data were available from 26 different dietary surveys carried out in 17 different European countries. These included infants from two surveys (two countries), toddlers from seven surveys (seven countries), other children from 15 surveys (13 countries), adolescents from 12 surveys

(10 countries), adults from 15 surveys (14 countries), the elderly from seven surveys (seven countries) and the very elderly from six surveys (six countries) (Appendix C, Table C.1).

Consumption records were codified based on the FoodEx classification system, which was developed by the DATA Unit in 2009 (EFSA, 2011a). Further details on how the Comprehensive Database is used are published in the Guidance of EFSA (2011b).

7. Exposure assessment

7.1. Previously reported human exposure assessments

Radovnikovic et al. (2013) studied the occurrence of nitrofuran marker metabolites in different foods of animal origin (see Section 5.1). SEM was the nitrofuran marker metabolite most frequently found in the tested samples. The exposure to SEM from prawns, salmon, honey and ovine liver was assessed for the Irish population using a probabilistic approach. The 95th percentile of the middle-bound dietary exposure was 0.04, 0.03 and 0.04 ng/kg b.w. per day, for adults, teenagers and children, respectively. The 95th percentile of the lower-bound dietary exposure was 0.005, 0.003 and 0.005 ng/kg b.w. per day, for adults, teenagers and children, respectively.

In addition, several national agencies evaluated the dietary exposure from shrimps/prawns in which AOZ had been detected (see Section 1.1).

7.2. Dietary exposure to nitrofuran marker metabolites for different scenarios

Only limited occurrence data on nitrofurans and their marker metabolites in food were available for this opinion (see Section 5.2). Therefore, the CONTAM Panel concluded that these data are too limited to carry out a reliable human dietary exposure assessment. Instead, the CONTAM Panel calculated the hypothetical human chronic dietary exposure using the RPA value of 1 µg/kg for two scenarios:

- Scenario 1A, in which foods of animal origin, excluding milk and dairy products, are contaminated with one nitrofuran marker metabolite at a concentration equal to the RPA level of 1 µg/kg. These are mainly meat and meat products, fish and fish products, eggs and egg products and honey.
- Scenario 1B, in which foods of animal origin, including milk and dairy products, are contaminated with one nitrofuran marker metabolite at a concentration equal to the RPA value of 1 µg/kg.

The CONTAM Panel noted that the foods of animal origin that are contaminated with nitrofuran marker metabolites, as reported in the EC database on residues of veterinary medicines and the RASFF database (see Section 5.2.2), are meat and meat products, fish and fish products, eggs and egg products and honey. These foods are included in scenario 1A. The CONTAM Panel emphasises that this scenario, in which all the consumed foods (meat and meat products, fish and fish products, eggs and egg products and honey) are considered to be contaminated with a nitrofuran marker metabolite at the RPA of 1 µg/kg, represents a highly unlikely situation.

Although raw milk is sampled and analysed for the presence of nitrofuran marker metabolites by some Member States in the EU (e.g. in 2013, 140 milk samples were analysed for the presence of AOZ, AMOZ, AHD and SEM by 13 Member States), no non-compliant results for nitrofuran marker metabolites in milk have been reported in the EC database and no notifications have been reported for milk or milk products in the RASFF database. However, the CONTAM Panel is aware that, in the past, furaltadone and nitrofurazone were used in dairy cattle, e.g. intramammary use to treat mastitis (Huber, 1982). In addition, use in sheep and goats to treat coccidiosis has been reported (Robertson, 1982). Therefore, the occurrence of nitrofurans and their marker metabolites in milk and dairy products owing to the illicit use of nitrofurans in individual diseased animals cannot be excluded. Milk

and milk products are included with the other foods of animal origin in scenario 1B. However, given that the illicit use of nitrofurans on a widespread basis in dairy animals is highly unlikely and the dilution effect of contaminated milk with uncontaminated milk that occurs at farm level (holding tanks), during transport (bulk tankers) and at the dairy processing plant (milk silos), scenario 1B is considered extremely unlikely.

The further scenario, in which all the foods included in scenarios 1A and 1B would be contaminated with all five nitrofuran marker metabolites, each at 1 µg/kg, is considered to be totally unrealistic, as samples containing multiple nitrofuran marker metabolites have not been found in the EC database on residues of veterinary medicines and have only rarely been found in the RASFF database. Therefore, this scenario was not considered.

To calculate a more refined dietary exposure to nitrofurans and their marker metabolites, the CONTAM Panel also considered that the above-mentioned foods are also consumed as part of composite dishes present in the FoodEx1 classification system. These are, for example, meat-based meals, fish-based meals, egg-based meals, soups and salads. In addition, in the case of infants and toddlers, meat and fish are normally consumed through ready-to-eat meals for infants and young children. For all the above-mentioned cases where it was clearly stated in the name of the meal that this was a mixture of meat or fish and other foods items (e.g. meat stew, fish and rice meal, fish and potatoes meal, fish and vegetables meal, ready-to-eat meal for children, meat and vegetables, etc.), a factor of 0.5 was applied, meaning that half of the quantity reported to be consumed was considered as referring to the meat or fish and the other half to the other food items present in the dish. All composite dishes and infants' foods were grouped based on their main ingredient.

For calculating the chronic dietary exposure to nitrofurans and their marker metabolites, food consumption and body weight data at the individual level were accessed in the Comprehensive Database. Exposure estimates were calculated for 26 different dietary surveys carried out in 17 different European countries. Not all countries provided consumption information for all age groups, and, in some cases, the same country provided more than one consumption survey.

Exposure was calculated by multiplying the occurrence concentration of 1.0 µg/kg for each food or food group with the consumption amount per kg b.w. separately for each individual in the database, calculating the sum of exposure for each survey day and then deriving the daily average for the survey period.

The mean dietary exposure and the high dietary exposure (95th percentile) to nitrofuran marker metabolites were calculated separately for each survey and age class using consumption data recorded at individual level from the Comprehensive Database (see Section 6). In accordance with the specification of the EFSA Guidance on the use of the Comprehensive Database (EFSA, 2011b), 95th percentile estimates for dietary surveys/age classes with fewer than 60 observations may not be statistically robust and therefore should not be considered in the risk characterisation.

As scenario 1B was considered extremely unlikely, this scenario was not used for the risk characterisation, and exposure estimates are presented in Appendix D, Table D.1. For scenario 1A, Table 5 provides per group the minimum, median and maximum of the mean and 95th percentile exposure values across dietary surveys. The mean chronic dietary exposure to nitrofuran marker metabolites ranges, for scenario 1A, from 1.7 to 8.0 ng/kg b.w. per day and the 95th percentile ranges from 3.5 to 13 ng/kg b.w. per day across dietary surveys and age classes.

Table 5: Summary statistics for the hypothetical chronic dietary exposure (ng/kg b.w. per day) to nitrofuran marker metabolites estimated by age class for scenario 1A.

Age class	Number of surveys	Scenario 1A[(a)]		
		Minimum	Median	Maximum
Mean dietary exposure				
Infants	2	2.7	–[(b)]	7.1
Toddlers	7	3.3	5.5	8.0
Other children	15	3.0	4.9	7.0
Adolescents	12	1.8	3.1	4.5
Adults	15	1.9	2.6	4.3
Elderly	7	1.8	2.3	2.5
Very elderly	6	1.7	2.2	2.6
95th percentile dietary exposure[(c)]				
Infants	1	–[(d)]	–[(d)]	–[(d)]
Toddlers	4	7.4	11	13
Other children	15	6.8	9.5	13
Adolescents	12	4.5	6.5	8.3
Adults	15	3.5	4.7	7.0
Elderly	7	3.9	4.0	4.6
Very elderly	5	3.6	3.8	4.4

The minimum, median and maximum of the mean and 95th percentile exposure values across dietary surveys in European countries are shown.

To avoid the impression of too high precision, the numbers for all exposure estimates are rounded to two figures.

(a): Scenario 1A contains foods of animal origin, excluding milk and dairy products, that are contaminated with one nitrofuran marker metabolite at a concentration equal to the RPA value of 1 µg/kg (meat and meat products, fish and fish products, eggs and egg products and honey).

(b): Not calculated; estimates available from only two dietary surveys.

(c): The 95th percentile estimates obtained from dietary surveys/age classes with fewer than 60 observations may not be statistically robust (EFSA, 2011b) and therefore are not included in this table.

(d): Estimates available from only one dietary survey: 11 ng/kg b.w. per day.

Besides arising from nitrofurazone use, SEM may occur in foods from other sources (see Section 3.3 and Appendix A). Except for carrageenan use, these other sources have been eliminated owing to changes in legislation or are covered by potential occurrence in foods of animal origin in scenario 1A. SEM can occur either naturally in carrageenan or as result of bleaching of carrageenan with a sodium hypochlorite solution (see Appendix A). Carrageenan—polysaccharides extracted from edible red seaweed—is authorised to be used as a food additive in a variety of food products, including those of non-animal origin, according to Regulation (EC) 1333/2008,[35] as amended. Therefore, the CONTAM Panel, in addition to the previous two scenarios which cover foods of animal origin only, calculated the chronic dietary exposure to SEM taking two additional potential sources of contamination into consideration: foods of non-animal origin and milk and dairy products for which carrageenan is authorised as an additive. For these scenarios, the level of contamination with SEM from carrageenan is set at the RPA level of 1 µg/kg or at concentrations calculated from maximum usage levels of carrageenan as a food additive and actual concentrations of SEM in carrageenan.

The chronic dietary exposure to SEM was calculated for the following scenarios:

- Scenario 2A, in which foods of non-animal origin for which carrageenan is authorised as an additive are contaminated with SEM at a concentration in the final food product equal to the RPA level of 1 µg/kg.
- Scenario 2B, in which foods of animal origin, excluding milk and dairy products, and foods of non-animal origin for which carrageenan is authorised as an additive are contaminated with SEM at a concentration equal to the RPA level of 1 µg/kg.

[35] Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 354, 31.12.2008, p. 16–33.

- Scenario 2C, in which foods of animal origin, including only those milk and dairy products for which carrageenan is authorised as an additive, and foods of non-animal origin for which carrageenan is authorised as an additive are contaminated with SEM at a concentration equal to the RPA level of 1 µg/kg.
- Scenario 2D, in which foods of animal origin, excluding milk and dairy products, are contaminated with SEM at a concentration equal to the RPA level of 1 µg/kg, and foods of non-animal origin and milk and dairy products, for which carrageenan is authorised as an additive, are contaminated with SEM at concentrations calculated from maximum usage levels of carrageenan and actual concentrations of SEM in carrageenan.

For scenarios 2A to 2D above, the following information and data have been used:

1. The detailed list of foods for which carrageenan is authorised to be used, according to Regulation (EC) 1333/2008, and their equivalent FoodEx1 food category.
2. Usage levels of carrageenan, reported by industry to EFSA through a public call for data,[36] contained in the EFSA additive database.
3. The concentration of SEM in the final food products calculated based on the mean SEM concentration of 65 µg/kg in carrageenan (AFC Panel, 2005; Appendix A) and the maximum usage level of carrageenan in different food categories.

Appendix E, Table E.1, presents the food categories for which carrageenan is authorised as an additive, the additive's classification code, the maximum permitted level, the equivalent food category in the FoodEx1 classification system of EFSA, the maximum usage level reported for the specific food category and the concentration of SEM in the final food.

Scenario 2A represents the hypothetical human chronic dietary exposure to SEM from use of carrageenan as a food additive in foods of non-animal origin, where all such foods would contain SEM at a level equal to the RPA of 1 µg/kg. Scenario 2B represents the hypothetical human chronic dietary exposure to SEM from use of carrageenan as a food additive in foods of non-animal origin (scenario 2A) and, in addition, from all meat and meat products, fish and fish products, eggs and egg products and honey that are contaminated with nitrofurazone marker metabolite (scenario 1A), where all such foods would contain SEM at a level equal to the RPA of 1 µg/kg. Scenario 2C is a further development of scenario 2B in that it represents the hypothetical human chronic dietary exposure to SEM from use of carrageenan as a food additive both in foods of non-animal origin and in milk products and, in addition, from all meat and meat products, fish and fish products, eggs and egg products and honey that are contaminated with nitrofurazone marker metabolite (scenario 1A), where all such foods would contain SEM at a level equal to the RPA of 1 µg/kg. The CONTAM Panel considers that all of these scenarios for human chronic dietary exposure to SEM represent highly unlikely situations. However, scenario 2A provides a basis for assessing the appropriateness of applying the RPA considered adequate to protect public health to commodities other than foods of animal origin and was therefore used for the risk characterisation.

Scenario 2D is a refinement of scenario 2C in that, rather than using the RPA of 1 µg/kg as a measure of the hypothetical human chronic dietary exposure to SEM from use of carrageenan as a food additive in foods of non-animal origin and in milk products, the contamination of these foods with SEM is considered to be at concentrations calculated from maximum usage levels of carrageenan and actual concentrations of SEM in carrageenan. This scenario also includes the hypothetical human chronic dietary exposure to SEM from all meat and meat products, fish and fish products, eggs and egg products and honey that are contaminated with nitrofurazone marker metabolite at a level equal to the RPA of 1 µg/kg. The CONTAM Panel, therefore, while considering scenario 2D to represent a highly unlikely situation with respect to human chronic dietary exposure to SEM from all food sources,

[36] Call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Published: 27 March 2013. Deadline 15 September 2013. Available at: http://www.efsa.europa.eu/en/data/call/130327.htm

considers it to be a less extreme scenario than scenario 2C. As the exposure to SEM from dairy products in which carrageenan is used is not covered by scenarios 1A or 2A, the CONTAM Panel also considered scenario 2C in the risk characterisation to evaluate the RPA of 1 µg/kg.

All exposure scenarios that were considered for SEM are summarised in Table 6.

Table 6: Exposure scenarios considered for the nitrofuran marker metabolites

Scenario	Sources of contamination					
	Foods of animal origin, excluding milk and dairy products	**Milk and dairy products**	**Milk and dairy products for which carrageenan is authorised**	**Milk and dairy products for which carrageenan is authorised**	**Foods of non-animal origin for which carrageenan is authorised**	**Foods of non-animal origin for which carrageenan is authorised**
	mm[(a)]: 1 µg/kg	**mm: 1 µg/kg**	**SEM[(b)]: 1 µg/kg**	**SEM: calculated from use**	**SEM: 1 µg/kg**	**SEM: calculated from use**
1A	x					
1B	x	x				
2A					x	
2B	x				x	
2C	x		x		x	
2D	x			x		x

mm: marker metabolite; SEM: semicarbazide.
(a): Concentration of one nitrofuran marker metabolite.
(b): Concentration of SEM.

Table 7 summarises the hypothetical chronic dietary exposure (ng/kg b.w. per day) to nitrofuran marker metabolites estimated by age class for scenarios 2A and 2C. For scenario 2A, the mean chronic dietary exposure to nitrofuran marker metabolites ranges from 2.3 to 41 ng/kg b.w. per day and the 95th percentile ranges from 5.9 to 63 ng/kg b.w. per day across dietary surveys and age classes. For scenario 2C, the mean chronic dietary exposure to nitrofuran marker metabolites ranges from 5.5 to 55 ng/kg b.w. per day and the 95th percentile ranges from 9.1 to 80 ng/kg b.w. per day across dietary surveys and age classes.

The exposure estimates for scenarios 2B and 2D are shown in Appendix F.

Table 7: Summary statistics for the hypothetical chronic dietary exposure (ng/kg b.w. per day) to nitrofuran marker metabolites estimated by age class for scenarios 2A and 2C.

Age class	Number of surveys	Scenario 2A[(a)]			Scenario 2C[(b)]		
		Min	Median	Max	Min	Median	Max
Mean dietary exposure							
Infants	2	4.2	–[(c)]	7.4	12	–[(c)]	23
Toddlers	7	4.6	14	41	17	29	55
Other children	15	11.0	19	31	18	28	42
Adolescents	12	6.3	9.8	15	11	16	19
Adults	15	3.3	5.7	13	6.4	9.6	16
Elderly	7	2.3	4.2	6.9	5.5	7.8	10
Very elderly	6	3.0	4.2	7.1	5.5	7.8	10
95th percentile dietary exposure[(d)]							
Infants	1	–[(e)]	–[(e)]	–[(e)]	–[(f)]	–[(f)]	–[(f)]
Toddlers	4	23	36	62	43	61	80
Other children	15	24	36	63	35	47	76
Adolescents	12	14	21	31	22	29	35
Adults	15	7.4	14	25	12	18	30
Elderly	7	5.9	8.0	15	9.3	13	19
Very elderly	5	6.0	8.8	15	9.1	13	18

The minimum, median and maximum of the mean and 95th percentile exposure values across dietary surveys in European countries are shown.
To avoid the impression of too high precision, the numbers for all exposure estimates are rounded to two figures.
b.w.: body weight; Min: minimum; Max: maximum.
(a): Scenario 2A contains foods of non-animal origin for which carrageenan is authorised as an additive and are contaminated with semicarbazide (SEM) at a concentration in the final food product equal to the reference point for action (RPA) level of 1 µg/kg.
(b): Scenario 2C contains foods of animal origin, including only those milk and dairy products for which carrageenan is authorised as an additive, and foods of non-animal origin for which carrageenan is authorised as an additive, and are contaminated with SEM at a concentration equal to the RPA level of 1 µg/kg.
(c): Not calculated; estimates available from only two dietary surveys.
(d): The 95th percentile estimates obtained from dietary surveys/age classes with fewer than 60 observations may not be statistically robust (EFSA, 2011b) and therefore were not included in this table.
(e): Estimates available from only one dietary survey: 23 ng/kg b.w. per day.
(f): Estimates available from only one dietary survey: 77 ng/kg b.w. per day.

7.3. Non-dietary exposure

In humans, there is the potential for additional exposure to nitrofurans from licensed medicines via oral or topical administration (see Section 1.3.1).

8. Hazard identification and characterisation

8.1. Toxicokinetics

8.1.1. Introduction

Because of the relatively low serum and tissue levels of the unchanged molecules recovered in orally treated individuals, the oldest studies on the kinetics of nitrofurans used for therapeutic purposes in humans and in animals led to erroneous conclusions. With the exception of nitrofurantoin, long known to be extensively excreted via the urinary route, nitrofurans were believed to be poorly absorbed and mainly excreted in the faeces. Subsequent investigations with radiolabelled compounds and the development of more sophisticated analytical methods have helped to establish that nitrofurans are instead generally well absorbed and extensively biotransformed, with the resulting metabolites excreted by both the biliary and the urinary routes (White, 1989). Accordingly, it became apparent that monitoring of nitrofuran residues in livestock based on the identification of the parent compounds was not efficient for surveillance purposes, because of the short *in vivo* half-lives of nitrofuran compounds and their rapid degradation in tissues post mortem (Nouws and Laurensen, 1990). More recent investigations, therefore, have concentrated on the study of nitrofuran biotransformations as the main

determinants of their adverse effects (Rao and Mason, 1987; Dalvie et al., 2002; Boelsterli et al., 2006) and the identification of metabolites to be used as proof of nitrofuran exposure in food-producing species (Radovnikovic et al., 2011) and in animal production (McCracken et al., 2005a; McCracken and Kennedy, 2007). In contrast with their parent compounds, it was found that residues of nitrofuran metabolites may persist for several weeks in edible tissues owing to extensive protein binding and are therefore suitable for residue monitoring purposes (Vass et al., 2008c).

There is sparse information about the biotransformation of nitrofurans and so it is difficult to provide a general overview of the metabolic fate of the nitrofurans considered in this opinion. Some species-related (Vroomen et al., 1990) and compound-related (Hoogenboom et al., 1994) differences in their biotransformation pathways have been reported.

It is generally accepted that the metabolic degradation of nitrofurans involves the reductive biotransformation of the 5-nitrofuran ring, which is quantitatively the most important reaction. In addition, unlike the nitroaromatic compounds, in which the ring systems are generally refractory to metabolic cleavage, the nitroheterocyclic rings are more prone to ring opening resulting in the formation of open-chain metabolites (Kedderis and Miwa, 1988).

Like other nitroaromatic compounds, 5-nitrofurans may undergo a one-electron and a two-electron reduction. The one-electron pathway leads to the formation of the nitroanion radical, which has been demonstrated by electron paramagnetic resonance spectroscopy using spin traps; nitroanion radicals may also be formed in the presence of catecholamine neurotransmitters and ascorbic acid (Rao and Mason, 1987; Lax and Kukolich, 1992; Rossi et al., 1996). The nitroanion intermediate may then have two different fates. Under aerobic conditions (Figure 2A) it can react, in a futile cycle, with molecular oxygen being oxidised back to the parent compound and producing superoxide anion, which, in turn, may generate other reactive oxygen species (ROS) (Boelsterli et al., 2006; Aracena et al., 2014). By contrast, under anaerobic conditions, or in tissues with low oxygen tension, such as the renal inner medulla and urinary bladder (Aperia and Leebow, 1964), the dismutation of the nitroanion radical may occur yielding a nitroso-derivative (Figure 2B), which may be further reduced to a hydroxylamino- and amino-derivative and then biotransformed to more stable metabolites (see below) (Zenser et al., 1981; Fau et al., 1992).

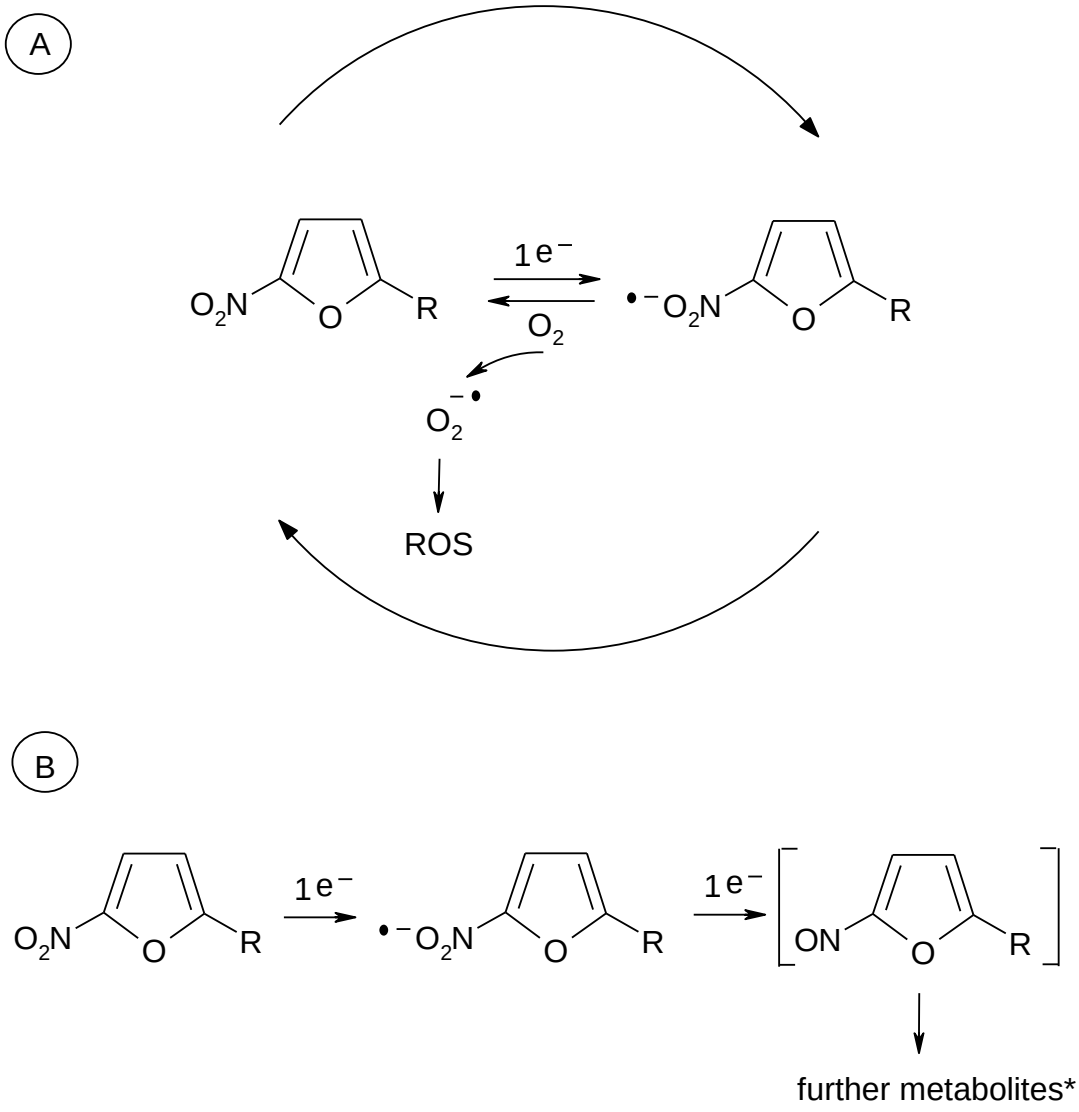


Figure 2: One-electron reduction of the nitrofuran ring. (A) Aerobic conditions: generation of the nitroanion radical and 'futile cycle' with the production of superoxide anions and of reactive oxygen species (ROS). (B) Anaerobic conditions: further reduction of the nitroanion radical to an unstable nitroso-derivative. *See Figure 3

The two-electron reduction (Figure 3) gives rise to the corresponding nitroso- and hydroxylamine unstable derivatives; the latter may be further reduced to the more stable amine, eventually subjected to N-acetylation, a detoxifying reaction (Boelsterli et al., 2006). Alternatively, the hydroxylamino intermediate may be dehydrated forming an unstable nitrile (cyano-) derivative that, in turn, may undergo a reduction of the double bond to form a stable open-chain metabolite (Figure 3). Other minor polar metabolites may also be produced (Dalvie et al., 2002). The enzymes involved in the 5-nitrofuran ring reduction may be divided into two classes, called type I and type II nitroreductases (NRs) (Peterson et al., 1979). Type I NRs are flavine mononucleotide (FMN)-binding proteins, mostly expressed in bacteria but more rarely in eukaryotes, that catalyse the two-electron reduction. Type II NRs are instead present in most mammalian tissues, contain FMN or flavine adenine dinucleotide (FAD) and mediate the one-electron reduction pathway (Ask et al., 2004). Scant data are available on the identification, the classification (type I or II) and the subcellular localisation of the specific enzymes mediating the reductive pathways. The microsomal enzyme nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome P450 (CYP) reductase is thought to primarily catalyse

this reaction, but xanthine oxidase, aldehyde dehydrogenase, mitochondrial NRs and nitric oxide synthases have also been implicated in this pathway (Boelsterli et al., 2006). Bacterial NRs expressed in a number of species found in the gut flora have also been shown to perform the 5-nitrofurans reduction (Hoogenboom et al., 1994; Ryan et al., 2011).

The oxidation of certain nitrofurans may also occur. Furaltadone is reported to undergo N-oxidation of the tertiary nitrogen of the morpholino ring in pig hepatocytes; interestingly, the resulting N-oxide derivative is not subjected to further metabolism to an open-chain nitrile derivative, which is instead produced upon the incubation of furaltadone with *S.* Typhimurium bacteria (Hoogenboom et al., 1994). Finally, the isolation of urinary 4-hydroxylated derivatives of a number of nitrofurans has also been reported in rats, rabbits, swine and chickens (Swaminathan and Lower, 1978; Streeter et al., 1988).

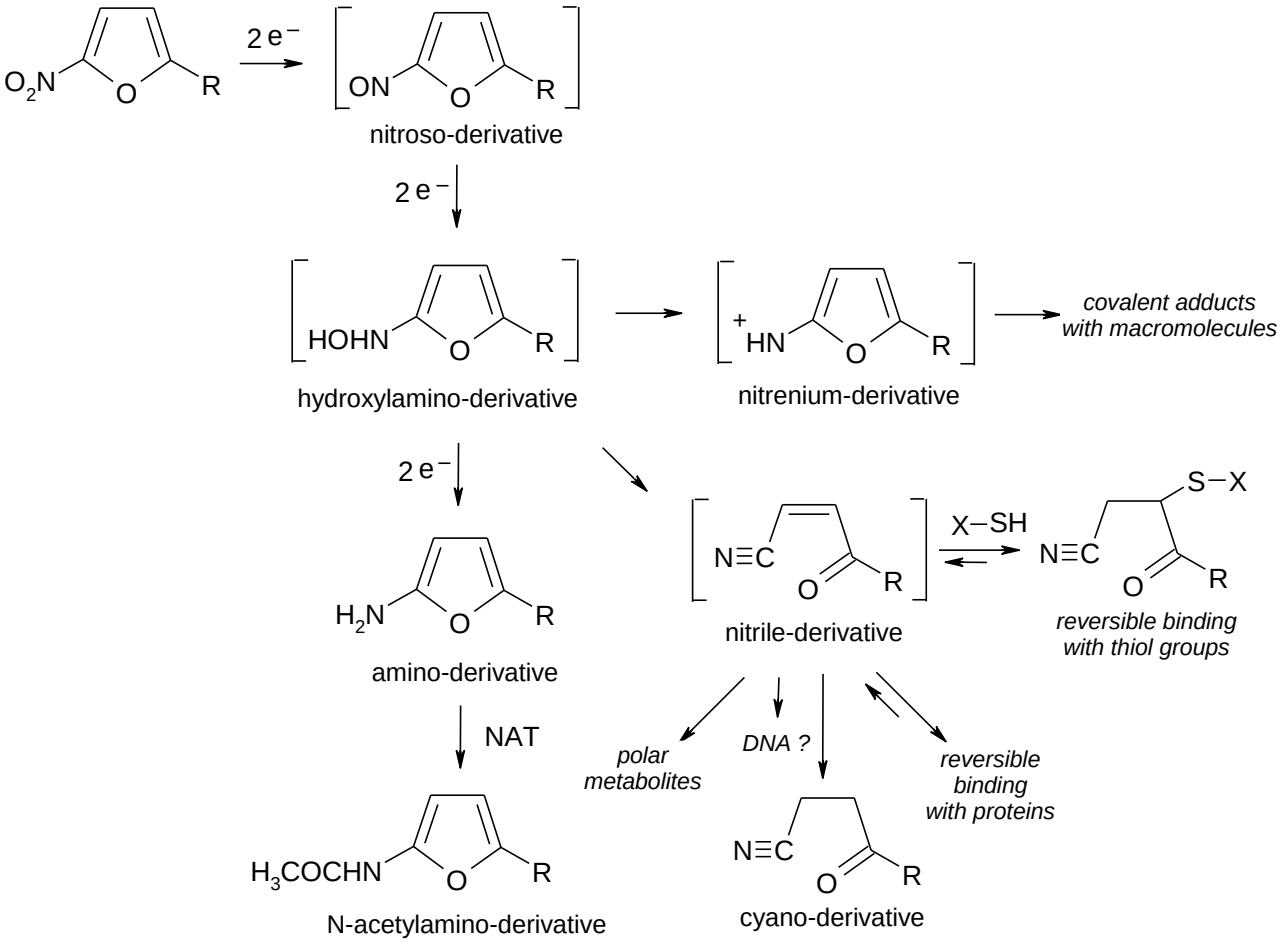


Figure 3: Two-electron reduction of the nitrofuran ring with generation of a stable (N-acetyl) amino-derivative or a number of reactive intermediates capable of reversibly binding to thiol groups and to proteins (NAT, N-acetyltransferases)

There is a general consensus that the nitroreductive pathway is clearly linked to the bioactivation of nitrofurans. Owing to their ability to form covalent adducts with macromolecules, unstable metabolites arising from the nitroreduction have been implicated in both the cytotoxic and the mutagenic effects of nitrofurans, as well as in the formation of the tissue-bound residues. All the metabolites giving rise to tissue-bound residues include an intact side-chain that is currently used as the marker residue for the nitrofurans under consideration (see Section 1.2). Finally, such metabolites may be released in the stomach of several species and be responsible for other adverse effects, such as monoamine oxidase

(MAO) inhibition (Yeung and Goldman, 1981). The exposure to nitrofurans results in an increased intracellular oxidative stress as reflected by the generation of superoxide anions and other toxic ROS. While the redox cycling of the nitroanion and/or the nitroso intermediates are mainly involved in the generation of oxidant species, other pathways such as the related alteration of the NADP+ to NADPH ratio and the depletion of glutathione (GSH) and of other critical thiol groups may participate in the enhancement of the nitrofuran-mediated oxidative stress (Boelsterli et al., 2006). GSH and other thiols may prevent the formation of certain reactive nitrofuran derivatives (Lax and Kukolich, 1992) and, under certain conditions, may also be depleted upon *in vitro* exposure to certain nitrofurans (Hoogenboom et al., 1992b; De Angelis et al., 1999). However, it should be noted that the nature of the reactive chemical species which bind to thiol groups has yet to be fully elucidated (and may vary with the different compounds). The formation of a GSH conjugate with the nitroanion derivatives of nitrofurazone and other 2-substituted nitrofurans could not be demonstrated *in vitro* (Polnaszek et al., 1984). Conflicting reports are available for nitrofurantoin, as the thiyl conjugate of the nitroanion metabolite detected by Nuñez-Vergara et al. (2000) by means of a spin trapping technique was not identified in other reports (Miller et al., 2002).

While the formation of GSH conjugates with nitroso- or hydroxylamino-derivatives cannot be excluded, *in vitro* studies with swine liver microsomes or hepatocytes suggest that, in the case of furazolidone, an open-chain acrylonitrile derivative identified as N-(4-cyano-2-oxo-3-butenylidene)-3-amino-2-oxazolidinone may be trapped by thiol-containing compounds such as GSH or mercaptoethanol (Vroomen et al., 1987b; Hoogenboom et al., 1992b) leading to the formation of unstable reversible conjugates. In fact, at physiological pH, the acrylonitrile derivative may be released and become covalently bound to cellular proteins forming protein adducts (Hoogenboom et al., 1992b). When incubated with thiols, the acrylonitrile derivative can be released from the protein again. The behaviour of such an intermediate is similar to that displayed by thiols in the reaction with α,β-unsaturated ketones, referred to as Michael and 'retro-Michael' reaction (Vroomen et al., 1988). It is unclear to what extent these unstable thiol adducts of nitrofurans play a role *in vivo*, as incubations of tissues from treated pigs with mercaptoethanol did not result in the formation of the mercaptoethanol conjugate (Vroomen et al., 1990).

At low pH, as in the stomach of many species, the azomethine bond (C=H) between the two parts of the molecule can be cleaved resulting in the release of the side-chain. Only in the case of pigs treated orally with furazolidone was it investigated and shown that the side-chain (in this case AOZ) is actually a metabolite occurring in the blood (Hoogenboom et al., 2002).

Finally, it has been demonstrated that some nitrofurans (nitrofurantoin and furaltadone) are able to cross the placental barrier in a variety of mammalian species (Buzard and Conklin, 1964; Perry and LeBlanc, 1967; Zhang et al., 2007), while data on the mammary excretion of the parent compounds are available for nitrofurantoin only. There is also evidence that nitrofuran metabolites may be excreted in (dairy) milk (Chu and Lopez, 2007) and eggs (McCracken and Kennedy, 2007).

8.1.2. Humans

Only very limited information is available concerning the pharmacokinetics of **furazolidone**. Ten healthy adults (weight, gender and age not specified) were orally administered 400 mg furazolidone in doses of 200 mg each for 21 days and the levels of the unchanged drug were determined in daily collected plasma and urine samples with an HPLC method. A wide range of plasma concentrations were reported (0.002–0.489 µg/mL) while the urinary excretion rates varied from 0.003 to 0.06 % of the daily dose. The authors conclude that furazolidone appears to be extensively metabolised in humans (Guinebault et al., 1981, as reported in White, 1989).

A single tablet of a commercial formulation containing 200 mg furazolidone (Giarlam™) was administered to 18 human volunteers (body weight range 55–100 kg); blood was collected 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 18 and 24 hours after dosing and the drug concentrations were measured by an HPLC method (LOD: 0.01 µg/mL; LOQ: 0.05 µg/mL). Kinetic data pointed to a good bioavailability of the

drug after oral administration, being detectable in serum as soon as 30 minutes after treatment and reaching a maximum serum concentration (C_{max}; 0.34 µg/mL) within the first 3 hours, with a $t_{1/2}$ of 4.87 hours (Calafatti et al., 2001).

The kinetics of **nitrofurantoin** were reviewed by Mandell and Sande (1985). It is rapidly and extensively absorbed by the gastrointestinal tract; slower absorption and urinary excretion rates and a lower incidence of adverse effects have been reported for the macrocrystalline form of nitrofurantoin than for the microcrystalline one (D'Arcy, 1985; Cunha, 1988; Brumfitt and Hamilton-Miller, 1998). Nowadays, mostly macrocrystalline nitrofurantoin is used (see section 1.3.1) but there are still a few formulations that contain the microcrystalline form. Only in a few studies the particle size was specified (Conklin and Hailey, 1969; Borsa et al., 1976; Rosenberg and Bates, 1976; Männistö, 1978). Microcrystalline nitrofurantoin has a particle size smaller than 10 µm (Rosenberg and Bates, 1976; Conklin and Hailey, 1969), approximately 10 µm (Borsa et al., 1976) or 4–15 µm (Männistö, 1978). The reported particle sizes of macrocrystalline nitrofurantoin were: 75–180 µm (Conklin and Hailey, 1969; Rosenberg and Bates, 1976), 80–180 µm (Borsa et al., 1976) or 90–200 µm (Männistö, 1978).

A study was designed in which six healthy human volunteers (weight range 62–80 kg) were administered 50 mg of nitrofurantoin (Ivadantin™) either as a tablet or as a slow intravenous (i.v.) infusion (Hoener and Patterson, 1981). The concentrations of both the parent compound and its amino-derivative arising from the complete reduction of the nitrofuran ring (aminofurantoin) were measured in plasma and urine specimens by means of an HPLC method. The drug kinetics after i.v. dosing could be described by a two-compartment open-body model with a calculated terminal plasma half-life of 58 ± 15 minutes, with about 60 % of the drug bound to plasma proteins. The average fraction of the drug that was excreted unchanged in the urine was 34 % when given orally and 47 % when administered i.v., reaching the peak at about between 105 and 170 minutes; the urinary excretion of the metabolite aminofurantoin amounted to about 1.2–1.4 % of the administered drug with no differences between the administration routes; a further minor urinary metabolite was tentatively identified as the N-acetylamino-derivative. Following the oral administration, C_{max} (428 ± 146 ng/mL) was reached after about 2 hours and slight differences in the kinetic parameters and in the bioavailability were observed between fasted and unfasted individuals. In contrast, both the bioavailability and the persistence of effective urinary concentrations were enhanced (from 20 to 400 %) in unfasted compared with fasted individuals administered with a single nitrofurantoin dose (100 mg), the largest differences being observed with dose forms exhibiting the lowest dissolution rate (i.e. tablets) (Rosenberg and Bates, 1976). A very limited drug excretion rate was reported in uraemic patients (Sachs et al., 1968), while genetic polymorphism of the ABCG2 C421A drug transporter had no apparent effects on plasma and urine kinetics in human volunteers orally administered with a single dose (100 mg) of the drug (Adkinson et al., 2008).

Scant information is available concerning the placental transfer of nitrofurantoin. In the only available report, 17 women in labour received 90 mg of the drug by i.v. infusion over a period of 30 minutes (Perry and Leblanc, 1967). At the time of delivery, samples of maternal venous blood, umbilical cord blood and, whenever possible, amniotic fluid were collected at different time points and nitrofurantoin concentrations were determined by means of a colorimetric method. The drug was found to readily cross the placental barrier reaching cord blood concentrations of the same order of magnitude of those measured in maternal blood (range < 1.0–9.8 µg/mL) with a ratio ranging from 0.4 to 2, but showing at the same time a rapid disappearance from fetal circulation. The overall usefulness of the study is limited by the poor sensitivity of the analytical method (LOD 1 µg/mL).

To investigate the transfer rate of nitrofurantoin in human milk, four healthy lactating women were treated with a single oral dose of macrocrystalline nitrofurantoin with food, and blood and milk samples were collected at different time intervals up to 12 hours after dosing (Gerk et al., 2001). Drug concentrations measured with an HPLC method (LOQ 0.01 µg/mL) were much higher in milk than in serum at any time point, displaying C_{max} values of 2.71 ± 0.65 versus 0.50 ± 0.14 µg/mL, which were reached in about 5 hours in both cases. As the milk–serum ratio was much higher (nearly 6) than that expected based on an *in vitro* model of passive diffusion, the authors concluded that nitrofurantoin is

actively excreted in human milk, as confirmed later by further *in vitro* studies (Gerk et al., 2003); in addition, they expressed health concerns for suckling infants younger than 1 month, or infants with a high frequency of G6PD deficiency or sensitivity to nitrofurantoin.

In conclusion, limited information is available on the kinetics of nitrofurans in humans. The oral bioavailability of the parent compound seems to be good, and in the case of nitrofurantoin was shown to be affected by drug particle size and possibly by fasting. No reliable data could be found on the biotransformative profile of all nitrofurans considered in this opinion.

8.1.3. Laboratory animals

Little information on the *in vivo* kinetics of **furazolidone** is available in the open literature. In rats administered with a single oral dose of 100 mg furazolidone/kg b.w., plasma levels of the nitrofuran measured with a colorimetric method amounted to 2.5 µg/mL 4 hours after treatment; after 48 hours, 3.2 % of the administered dose could be recovered in the faeces as the unchanged molecule using a microbiological method (Paul et al., 1960b). In a more recent study (Tatsumi et al., 1984), the excretion profile was investigated in rats using the labelled compound. In animals receiving a single oral dose of ^{14}C-furazolidone (10 mg/kg b.w.), 50 % of the radioactivity was recovered in faeces and urine collected over 7 days; the identification of the N-acetylamino-derivative in urine samples provided evidence of the formation of the amino-derivative as an intermediate metabolite of furazolidone in rats. In a further experiment in which the labelled compound was administered orally to rats at doses of 100 mg/kg b.w. for 4 days, an open-chain cyano-derivative (3-(4-cyano-2-oxobutylideneamino)-2-oxazolidone), an open-chain carboxylic derivative ((4-carboxy-2-oxobutylideneamino)-2-oxazolidone) and α-ketoglutaric acid were also identified in urine, accounting for 25 % of urinary radioactivity. Water-soluble unidentified metabolites accounted for the rest of the urinary radioactivity.

The *in vitro* metabolism of ^{14}C-furazolidone was characterised using rat liver S9 or *Escherichia coli* whole cell extracts or lysates under aerobic and anaerobic conditions (Abraham et al., 1984). In line with the results of the *in vivo* studies, in all cases, the major metabolite was the result of a reductive pathway and was unequivocally identified as an open-chain cyano-derivative (3-(4-cyano-2-oxobutylideneamino)-2-oxazolidone). It is also worth noting that a significant proportion of the radioactivity remained covalently bound to liver proteins. In a subsequent study investigating the metabolic fate of ^{14}C-furazolidone using rat liver microsomes, Vroomen et al. (1987b) found two main non-polar metabolites, i.e. the known open-chain cyano-derivative and a second one (2,3-dihydro-3-cyanomethyl-2-hydroxy-5-nitro-1α,2-di-(2-oxo-oxazolidin-3-yl)iminomethylfuro[2,3-b]furan), both resulting from the reduction of the nitrofuran under aerobic or anaerobic conditions. CYP was not involved in the generation of the above metabolites, which could also be produced upon incubation with purified NADPH-CYP reductase. The addition of GSH (2 mM) to the incubation mixture drastically decreased both the formation of the above-mentioned non-polar metabolites and the extent of the covalent binding to microsomal proteins, which was most likely due to (reversible) GSH interaction with unstable nitroso and hydroxylamino furazolidone derivatives.

Very little is known about the kinetics of **furaltadone** in laboratory species. In rats administered a single oral dose of 100 mg furaltadone/kg b.w., plasma levels of the nitrofuran measured with a colorimetric method amounted to 3.0 µg/mL 4 hours after treatment; after 48 hours, 3.4 % of the administered dose (138 mg/kg b.w.) could be recovered in urine, while faecal excretion was negligible (Paul et al., 1960b). To study the placental transfer of furaltadone, the drug was i.v. infused at doses ranging from 5 to 15 mg per hour for various lengths of time to M-F strain guinea pigs, New Zealand White rabbits, mongrel dogs or Hampshire sheep in their last trimester of pregnancy. A colorimetric method able to measure the nitrofurfurylidene moiety was used to quantify the nitrofuran in maternal and fetal plasma. The placental transfer of furaltadone could be demonstrated in all examined species with a plasma fetal–maternal ratio ranging from about 0.35 to about 0.47 (Buzard and Conklin, 1964). *In vitro* studies performed with rat liver homogenates pointed to an NADPH-mediated reductive biotransformation involving the nitro-group (Akao et al., 1971).

Buzard et al. (1961) studied the kinetics of **nitrofurantoin** in the rat. To investigate the site of absorption after oral administration, nitrofurantoin (25 or 100 mg/kg b.w.) was injected into different segments of the rat gastrointestinal tract, namely the stomach, small intestine, caecum and colon. After blood collection and nitrofurantoin analysis, it was concluded that the drug was rapidly and extensively absorbed in the small intestine and, to a minor extent, in the colon. A plasma half-life of 25 minutes could be measured following the i.v. dosing (25 mg/kg b.w.). As, after bilateral nephrectomy or ligation of the ureters, the half-life of nitrofurantoin increased to around 70 minutes, it was concluded that urinary excretion is an important elimination pathway. In this respect, a higher rate of tubular reabsorption (up to 10-fold) was noted in young (5- or 15-day-old) than in older (33- or 55-day-old) rats treated with 20 mg nitrofurantoin/kg b.w. (i.v.); the reason for this age-related difference resides in the lower urinary pH displayed by the young rats causing the shift of nitrofurantoin (a weak acid) towards the non-ionised form.

The bioavailability and the kinetic parameters of nitrofurantoin were investigated in rabbits (Watari et al., 1983). Two groups of male Japanese white rabbits (n = 16) were dosed with the drug (1.25 or 10 mg/kg b.w.) using one of the following routes: i.v., intraduodenal or oral administration. Plasma samples were collected before administration and over a 90-minute period after administration for the low-dose group and over a 2.5-hour period after administration for the high-dose group. Urine was collected 8 hours after administration. Nitrofurantoin was measured with a fluorometric method (LOD not reported). The plasma concentration–time course after i.v. dosing could be fitted by a one-compartment model. Plasma levels were significantly lower after oral administration with respect to the other routes. At the low dose, the C_{max} was 0.26 µg/mL after oral administration and around 1.55 µg/mL after the other routes of administration, whereas at the high dose it was 1.73 µg/mL after oral administration and approximately 14 µg/mL after the other routes of administration. The reason for the reduced bioavailability was attributed to a rapid drug decomposition in the stomach, probably due to the (reversible) cleavage of the azomethine bond under acidic conditions. Finally, the percentage of the unchanged drug that was excreted in urine varied depending on the route of administration and the administered dosage. While in the animals given 1.25 mg nitrofurantoin/kg b.w. it ranged from around 16 % (oral route) to around 50 % (other routes), at 10 mg/kg b.w. it amounted to about 20 %, irrespective of the administration route.

The presence of 4-hydroxynitrofurantoin was unequivocally demonstrated in urine samples from male Sprague–Dawley rats after a single oral dose of ^{14}C-nitrofurantoin (33 mg/kg b.w.); the amount of such urinary metabolite was found to sharply increase (up to about 16-fold) in 3-methylcholanthrene- or β-naphthoflavone-induced rats over phenobarbital pretreated or untreated animals (Jonen et al., 1980), pointing to the involvement of the CYP1 family in the 4-hydroxylation of the drug.

The role of caecal microflora in the *in vivo* nitroreduction of nitrofurantoin was demonstrated by Rowland et al. (1983). A negligible rate of nitrofurantoin reduction was found upon the incubation with caecal suspensions from rats administered a diet supplemented with carrageenan (50 g/kg diet) with respect to rats offered a basal diet; such an effect was attributed to the strong decrease of gut bacterial populations observed in animals exposed to the food additive.

The *in vitro* metabolism of nitrofurantoin in rats was characterised using an HPLC method by Aufrère et al. (1978) who demonstrated that, under anaerobic conditions, the drug was rapidly biotransformed in liver and small intestinal wall preparations and by colon or caecum content as well, but only to a lesser extent in kidney and lung. The main metabolite was the open-chain acrylonitrile derivative resulting from the nitroreduction followed by the opening of the furan ring. In another *in vitro* study, the one-electron reduction of nitrofurantoin by rat liver mitochondria with the generation of the nitroanion radical and the superoxide anion could be demonstrated by direct electron spin resonance spectroscopy and spin trapping experiments, respectively (Moreno et al., 1984). An increasing body of literature (Oo et al., 2001; Merino et al., 2005, 2010; Wang and Morris, 2007; Wang et al., 2008) indicates that, based on both *in vitro* and *in vivo* studies using knock-out mice, nitrofurantoin is a substrate of the transmembrane breast cancer resistance protein (BCRP/ABCG2), a member of the ATP-binding cassette family of transporters affecting the kinetics of several drugs, toxins and

food/feed components. Remarkable differences in oral bioavailability (almost a 2-fold increase), hepatobiliary excretion rate (nearly abolished) and milk–plasma ratio (80-fold decrease) were reported between BCRP–/– and wild-type nitrofurantoin-treated mice (Merino et al., 2005). The effects of the co-administration of nitrofurantoin and a mixture of two soy isoflavones known for their inhibitory effects on drug transporters (Okura et al., 2010), namely genistein and daidzein, were investigated in mice (Merino et al., 2010). Wild-type mice were orally dosed with nitrofurantoin alone (20 mg/kg b.w.) or nitrofurantoin plus the isoflavones (both at 100 mg/kg b.w.). The isoflavone addition resulted in both a statistically significant increase in nitrofurantoin plasma concentration at 30 minutes (1.7-fold) and a strong inhibition of the BCRP/ABCG2-mediated nitrofurantoin secretion in milk, as reflected by the lower milk–plasma ratio (7.1 ± 4.2 versus 4.2 ± 1.6). Mice with isoflavones also showed a remarkable reduction in nitrofurantoin bile levels (3.7 ± 3.2 versus 8.8 ± 3.4 µg/mL). The authors concluded that the co-administration of other BCRP/ABCG2 substrates may significantly affect the *in vivo* kinetics of nitrofurantoin.

Limited information is available concerning the kinetics of **nitrofurazone** in experimental species. In rats administered with a single oral dose of 100 mg nitrofurazone/kg b.w., plasma levels of the nitrofuran measured with a colorimetric method amounted to 4.5 µg/mL 4 hours after treatment; after 48 hours, 4.6 % of the administered dose could be recovered in the faeces as the unchanged molecule using a microbiological method (Paul et al., 1960a). In a further study, the excretion products recovered in the urine of nitrofurazone-treated rats (dosage and route of administration not specified) were tentatively identified as hydroxylamino or aminofuraldehyde semicarbazone (Paul et al., 1960b). The oral absorption and the urinary and biliary excretion rates of ^{14}C-nitrofurazone were investigated by Tatsumi et al. (1971) in rats administered with a single dose of the nitrofuran (100 mg/kg b.w.). After 48 hours, the percentage of recovered radioactivity amounted to approximately 60 % in urine, 27 % in the bile and 12 % in the gastrointestinal tract. Because, as determined by reverse dilution analysis or spectrophotometry, very little radioactivity was attributable to the unchanged compound in all examined matrices (less than 0.3 %), it was concluded that oral nitrofurazone is extensively absorbed and biotransformed in rats. To characterise the *in vivo* reduction of nitrofurazone, male conventional or germ-free Sprague–Dawley rats were treated with 0.13 mg ^{14}C-nitrofurazone/kg b.w. by gavage and the urinary metabolites analysed by HPLC (Tin-Chuen et al., 1983). The main reduced metabolite was identified as the open-chain stable cyano-derivative 4-cyano-2-oxobutyraldehyde semicarbazone; the amount of such a metabolite in urine from conventional rats was approximately twice that measured in germ-free rats, suggesting an important contribution of the enteric flora in the reductive pathway of nitrofurazone.

In experiments carried out with the isolated perfused rat liver (Hoener, 1988), the nitrofurazone-mediated oxidative stress induced by both the nitroanion redox cycling and the generation of further reactive unstable reduced metabolites (see Section 8.1.1) could be inferred by the marked increase in biliary glutathione-disulphide (GSSG) along with a marked decline of tissue GSH levels. Using ^{35}S-methionine, a GSH adduct with nitrofurazone (or its metabolites) could be identified by means of HPLC.

In conclusion, only sparse information is available for laboratory species concerning the *in vivo* biotransformation profile of the nitrofurans considered in the present opinion and the form in which they are deposited in tissues.

8.1.4. Biotransformation in livestock, horses and fish

Prior to the discovery of protein-bound residues and the development of a method to detect them by releasing the side-chain under acid conditions (Hoogenboom et al., 1991c), studies focused primarily on the parent compound and, in some cases, on metabolites such as the open-chain cyano metabolite. In general, these studies show rather low levels of the parent compound, if it is detectable at all. This is quite different for the bound residues and the side-chains, which show rather high levels and long half-lives. For the current opinion, the focus was primarily on studies to determine these bound residues and their kinetics.

8.1.4.1. Ruminants

Limited information is available on the transfer of nitrofurans or their metabolites into milk. In a study by the United States Department of Agriculture (USDA), Smith et al. (1998) applied radiolabelled nitrofurazone (label in the carbon of the azomethine bond) to dairy cows with intramammary application, intrauterine application or application via the eyes (single doses of 65.6 mg (intramammary) or 36.8 mg (intrauterine), or 4 days of 2.1 mg on the surface of the eyes), in order to determine to what extent this off-label use would result in residues in milk. In all cases, a small part of the label was excreted via the milk; in the case of the intramammary infusion, 1.25 and 1.09 % of the label was excreted in the dosed and undosed quarters, respectively; in the case of the intrauterine treatment, 0.45 % was excreted; and, in the case of the eye treatment, 0.45 % was also excreted. All three animals were slaughtered on day 6 and the radiolabel was detected in all tissues examined, with the highest levels in liver and kidney and in the tissues where the drug was applied. Levels of the parent drug were determined in milk and accounted for only a small part of the radiolabel. The highest level was 11.4 µg/kg in milk from the treated quarter collected between 0 and 12 hours after the treatment, compared with 2.8, 0.7 and 0.3 µg/kg in milk collected between 12 and 24 hours, between 24 and 36 hours and between 36 and 48 hours, respectively. In the case of the intrauterine treatment, the parent drug was detected in the milk collected between 0 and 12 hours only, at a level of 1.2 µg/kg. It was apparently not possible to detect the released side-chain, but the method was not described.

Chu and Lopez (2007) developed a method to detect the released side-chains from residues in milk and treated one cow orally with one dose of a cocktail of nitrofurazone, furazolidone, furaltadone (each 648 mg) and nitrofurantoin (3.2 g). Initial levels in milk collected during the first 12 hours were around 54, 46, 33 and 33 µg/kg for AMOZ, AHD, AOZ and SEM, respectively. Levels rapidly decreased to below detection limits (0.2 µg/kg) at 72 hours.

The milk excretion of nitrofurantoin and its modulation by isoflavones, which are known inhibitors of the efflux protein BCRP/ABCG2 (Okura et al., 2010), were investigated by Pérez et al. (2009) in sheep. Lactating ewes (weight range 70-75 kg) were offered a standard diet or a diet without isoflavones and all animals received a single dose of nitrofurantoin (20 mg/kg b.w.) by gavage with or without the prior oral administration of genistein and daidzein, each at the dose of 10 mg/kg b.w. Milk samples were collected up to 24 hours after dosing and nitrofurantoin concentrations were measured by an HPLC/UV method. While the time to peak concentration (T_{max}) values (range 1.6-3.5 hours) did not display statistically significant changes, much higher C_{max} values ($P<0.05$) were recorded in the group fed the isoflavone-deprived diet (18.3 ± 14.2 µg/mL) with respect to those measured in animals fed the isoflavone-adequate diet without (5.2 ± 3.9 µg/mL) or with (2.6 ± 1.5 µg/mL) genistein and daidzein administration. The results indicate that in sheep, nitrofurantoin is excreted in the milk and that this process is very likely mediated by BCRP/ABCG2.

8.1.4.2. Pigs

Vroomen et al. (1987a) treated 6-month-old pigs (n = 10) with medicated feed containing 300 mg **furazolidone**/kg for 10 days. Animals were killed 2 hours or 1, 3, 7 or 14 days after the last treatment. Blood, urine and various tissues were analysed for the parent compound and for the only known metabolite, the open-ring cyano metabolite, using HPLC-UV. Levels of the parent drug reached plasma levels up to 80 µg/L but decreased to non-detectable levels 7 hours after treatment. Very similar levels and patterns were observed for the cyano-metabolite, reaching the highest levels of 35 µg/L. In muscle, kidney and liver, the parent compound could not be detected (< 2 µg/kg). The cyano-metabolite was detected in muscle, but only 2 hours after the last treatment. These studies showed that the treatment of pigs with furazolidone did not result in detectable residues of the parent compound.

In order to study the fate of furazolidone in pigs, Vroomen et al. (1986) carried out a study with two piglets treated orally with 75 mg per day of the drug radiolabelled in the AOZ side-chain. This was done in accordance with the former therapeutic dose. One piglet was killed after 10 days of treatment, and the other one after a withdrawal period of 7 days. Various tissues were collected and examined for

the radiolabel. In the piglet killed just after the last treatment, the amount of the ^{14}C-label, expressed as furazolidone equivalents, was 6 000 µg/kg in muscle, 33 000 µg/kg in kidney and 30 000 µg/kg in liver. In the other piglet, levels were considerably lower, being 2 000 µg/kg in muscle, 3 000 µg/kg in kidney and 3 000 µg/kg in liver. Tissues were examined for the presence of furazolidone, showing that most of the radiolabel was present as unknown metabolites. A major part of the radiolabel could not be extracted from the tissues with various organic solvents and was therefore called protein-bound residues. This fraction was 14, 9 and 35 % for liver, kidney and muscle, respectively, from the piglet killed directly after the last treatment, and 27, 31 and 56 % for liver, kidney and muscle, respectively, from the piglet killed 7 days after the last treatment. It should be stressed that the extraction included an extraction with water, potentially resulting in the loss of small proteins/peptides and as such an underestimation of the actual fraction of bound residues. The same may occur with organic solvents that are not able to precipitate all the proteins.

Bound residues could be drug-like adducts, but it was also argued that the drug was very extensively metabolised and that radiolabelled fragments were incorporated into amino acids and subsequently proteins. However, muscle and liver tissues of this experiment were used by Hoogenboom et al. (1991c) to show that, similar to treated pig hepatocytes, AOZ could also be released from muscle and liver samples of animals treated with the drug. Levels of AOZ released from the liver were 23 and 14 % of the bound residues for the pigs killed just after the last drug treatment and 7 days later, respectively.

Hoogenboom et al. (1992a) performed a follow-up study with 3-month-old pigs (n = 10) to further study the fate of the bound residues. Animals were given the therapeutic dose through the feed (300 mg/kg feed) for 7 days. Withdrawal periods of 0, 1, 2, 3 and 4 weeks were applied. As observed previously, residue levels of the parent compound were non-detectable. However, in this study, the newly developed method for the marker residue, AOZ, was applied on samples from these pigs showing a gradual decrease in the levels. However, AOZ was still detected in liver, kidney and muscle samples from pigs killed 4 weeks after the last treatment at levels of 41, 7 and 10 µg/kg, compared with 993, 600 and 124 µg/kg, respectively, when slaughtered without any withdrawal period. When plotted on a log scale, this study showed a linear decrease in the levels of released AOZ. As also observed in the previous study with piglets by Vroomen et al. (1986), the decrease in muscle tissue seemed slower than that in liver and kidney.

Gottshall and Wang (1995) performed a study with six pigs (40–45 kg) treated with furazolidone ^{14}C-labelled in both the nitrofuran and the AOZ rings. The drug was orally applied for 14 days and animals were slaughtered 10 hours, 21 days and 45 days after the last treatment. Initial levels of residues (10 hours) in liver, kidney and muscle, expressed in furazolidone equivalents, were 41, 34, and 13 mg/kg and decreased to 4.4, 3.4 and 3.3 mg/kg, respectively, after 21 days and to 2 mg/kg in all tissues after 45 days. In addition, in this study, tissues were analysed for the marker residue AOZ, showing that the fraction of AOZ that could be released from the residues decreased over time; for example, in liver, the fraction started at 18 % at 10 hours and decreased to 13 % at 21 days and to 9 % at day 45. Nevertheless, levels of AOZ released from liver were 3 404 µg/kg at 10 hours, 247 µg/kg at day 21 and 79 µg/kg at day 45. In muscle, these levels were 1 291, 110 and 62 µg/kg, respectively, confirming that the difference between liver and muscle gets smaller after time, possibly as a result of a higher turn-over of tissue proteins in liver.

In order to validate their improved analytical method, Leitner et al. (2001) treated two pigs for 3 days with a therapeutic dose of either furazolidone or furaltadone. Animals were slaughtered immediately after the last treatment and analysed without prior extraction. Levels of AOZ and AMOZ in muscle meat were 100 and 30 µg/kg, being lower than observed before, possibly owing to the short treatment.

Cooper et al. (2005) treated 8-week-old piglets for 10 days with feed medicated with furazolidone, furaltadone, nitrofurantoin or nitrofurazone at a dose of 400 mg/kg feed. Animals were slaughtered after a withdrawal period of 0, 1, 2, 3, 4 or 6 weeks and liver, kidney and muscle tissue was collected. Tissues were first extracted with organic solvents and the precipitate containing the bound residues

was treated with hydrogen chloride to release the respective side-chains. Levels were determined with LC-MS. With the exception of some low levels at time 0, no parent compounds could be detected. In the case of furazolidone, initial levels of AOZ released from liver, kidney and muscle were around 2 000, 1 000 and 400 µg/kg, respectively, being in a similar range as that observed previously. Levels decreased gradually to about 40 µg/kg in liver and muscle and 10 µg/kg in kidney after 6 weeks. Depletion half-lives varied between 7 days for liver and kidney and 12 days for muscle. In the case of furaltadone, initial levels of AMOZ released from liver, kidney and muscle were around 5 000, 3 000 and 1 700 µg/kg, respectively—all higher than for furazolidone, and also higher when corrected for molecular weight. Levels showed a similar decline as for furazolidone during the withdrawal period, again with relatively high levels after 6 weeks. Nitrofurantoin showed somewhat lower levels of AHD released from the various tissues, with levels just below 10 µg/kg after 6 weeks, but again showing a similar decline. In the case of nitrofurazone, initial levels were relatively high, especially in muscle and when expressed on a molar basis, showing initial levels of 2 000 and 300 µg/kg, respectively, after 6 weeks. This depletion from muscle was much slower than for the other nitrofurans, which is also shown by the long half-life of 15 days.

Liu et al. (2010a) treated young piglets (15–18 kg) for 7 days with medicated feed containing 400 mg/kg furazolidone, followed by a withdrawal period of 0.5, 7, 21, 35, 56 or 63 days. In addition to liver, kidney and muscle, plasma and urine were also collected and analysed to examine the potential use of these matrices to predict the levels in tissues. Tissues were not extracted prior to analysis, and an immunoassay was applied to quantify AOZ after derivatisation. Half a day after the last treatment, levels in liver, kidney and muscle were around 2 000, 1 000 and 700 µg/kg, respectively, which are very similar to those observed in other studies. The decrease in these levels was relatively slow and, after 63 days, AOZ was still detectable at levels around 1 µg/kg. Interestingly, levels in both plasma and urine showed a very good correlation with those in tissues, indicating the gradual release of AOZ-containing peptides and excretion into the urine. As such, urine and plasma can be used to determine the treatment of animals even after a prolonged time.

8.1.4.3. Poultry

Transfer to eggs

Various studies have shown that nitrofurans in poultry feed may result in residues in eggs. McCracken et al. (2001) were the first to also include the releasable side-chains in these studies. Laying hens were fed for 11 days with a feed containing furazolidone at 400 mg/kg, which was the former therapeutic level. Within 3 days, the levels of furazolidone reached a plateau level around 400 µg/kg, very similar to the level of AOZ. On a molar base, the parent compound could explain only part of the AOZ. When ending the treatment, the levels of furazolidone dropped to around 4 µg/kg after 4 days, while AOZ was still detectable at levels around 3 µg/kg after 21 days. Most of the furazolidone was detected in the egg white during the treatment, whereas, for AOZ, levels were similar in egg white and yolk. However, 11 days after the treatment, AOZ levels in the yolk were three-fold higher, which might be related to the fact that production of yolk requires much more time than the albumen.

In a second study, McCracken and Kennedy (2007) also included nitrofurazone, nitrofurantoin and furaltadone as well as furazolidone. Hens were fed for 1 week with medicated feed containing 300 mg/kg of either one of these four nitrofurans. Yolk of eggs collected for 2 days after the treatment showed levels for furazolidone, furaltadone, nitrofurantoin and nitrofurazone of, respectively, 1.82, 1.83, 0.28 and 4.18 nmol/g (corresponding to, respectively, 410, 593, 67 and 828 µg/kg). Levels of the side-chains AOZ, AMOZ, AHD and SEM were, respectively, 4.68, 2.93, 1.47 and 15.13 nmol/g (corresponding to, respectively, 477, 589, 222 and 1 135 µg/kg), and again, on a molar base, the levels were much higher than for the parent compounds. In egg white, the levels for the parent compounds were slightly lower, except for nitrofurazone where the difference was much larger. A similar difference between egg yolk and egg white was also found for the side-chains. Both the parent compounds and side-chains were also detected in the shell, and levels were particularly high for SEM

(24 nmol/g). When compared with the previous study, levels for furazolidone and AOZ were in a similar range.

Cooper et al. (2008a) fed broiler breeder hens with feed containing nitrofurazone at levels of 0.03, 0.3, 3, 30 or 300 mg/kg for a period of 16 days. Levels of the parent compound in whole eggs increased rapidly, reaching plateau levels within 4 days, being around 1, 7, 70 and 700 µg/kg for the four highest dose groups. A similar pattern was observed for SEM, but levels were slightly higher. Hens fed the highest dose were transferred to clean feed, resulting in a decrease in the levels of nitrofurazone and SEM with half-lives of 1.1 and 2.4 days, respectively. About 75 % of SEM and 60 % of nitrofurazone was present in the yolk. About 16 % of the SEM in yolk could be attributed to the parent compound, compared with 36 % in the case of the albumen (overall about 28 %). These data all refer to total SEM. Tissue-bound SEM was determined in whole eggs of the 3 and 30 mg/kg dose groups, showing that about 30 % of the SEM was present in the non-extractable part.

McCracken et al. (2005b) also studied the transfer of metabolites of furazolidone, nitrofurazone, nitrofurantoin and furaltadone from breeder hens via the eggs to the progeny. Hens received feed with 400 mg/kg of the drugs, and eggs that were collected after more than 1 week on the drugs were bred. Young chicks were slaughtered at various ages; at day 1, liver levels of AOZ, AMOZ, AHD and SEM of, respectively, 7, 11, 3 and 27 µg/kg and muscle levels around, respectively, 5, 1, 3 and 33 µg/kg were found. Levels in both liver and muscle rapidly declined to below 1 µg/kg, but remained detectable up to 10 to 42 days, depending on the nitrofuran. In general, tissue levels were much lower than those in eggs, being 573, 1 004, 501 and 825 µg/kg for AOZ, AMOZ, AHD and SEM, respectively, and were comparable to those from the previous study (McCracken et al., 2001). This study implies that there is carry-over via the eggs but that only part of the residues end up in the progeny.

Barbosa et al. (2012) fed laying hens with medicated feed containing either 150 mg/kg of furaltadone or 100 mg/kg of nifursol for a period of 3 weeks followed by 3 weeks on clean feed. Eggs were analysed for both the parent compounds and the side-chains AMOZ and DNSH (without prior extraction of the proteins with solvents). As shown before, the parent compounds could be detected during the treatment but not at the end of the first week on clean feed. Levels of furaltadone in egg yolk and egg white were 384 and 242 µg/kg, and of nifursol were 271 and 83 µg/kg, respectively, suggesting a more selective excretion of nifursol into yolk. Levels of AMOZ in egg yolk/egg white during the treatment were 629/494 µg/kg and decreased to 29/31, 5.2/3.2 and 3.1/2.9 µg/kg at the end of 1, 2 or 3 weeks on clean feed, respectively. This shows that, during the treatment, the parent compound was responsible for a maximum of one-third of the detected side-chain, taking into account the difference in molecular weights. For nitrofurazone, the levels of the side-chain DNSH in egg yolk/egg white were 1 595/220 during the treatment and decreased to 65/12, 2.1/ND (not detectable) and ND/ND at the end of 1, 2 or 3 weeks on clean feed, respectively. In this case the parent compound could explain 11 and 25 % of DNSH in egg yolk and white, respectively, which is even less than in the case of furaltadone. When given feed with a mixture of 15 mg/kg furaltadone and 10 mg/kg nifursol, levels of parent compounds and side-chains in eggs were roughly 10-fold lower, with the exception of DNSH in yolk, which showed a higher ratio of almost 20-fold.

Transfer to meat and tissues

Zuidema et al. (2005) investigated the presence of tissue-bound residues of nifursol in 2-week-old broilers. A medicated feed containing 50 mg/kg nifursol was supplied for 7 days, followed by a switch to clean feed. Animals were slaughtered 0, 3, 7, 14 or 21 days after the switch. The parent compound could be detected in bile and plasma at days 0 and 3, but not in any of the tissues collected. Tissues were analysed for the releasable side-chain DNSH after extraction with organic solvents. DNSH levels were 900, 2 000 and 225 µg/kg in liver, kidney and muscle tissue. The extracted fraction was also analysed, showing levels of about 10 % of the non-extractable part. After 21 days, DNSH could still be detected at levels around 10 µg/kg in liver and kidney, just below 1 µg/kg in muscle and around 3 µg/kg in plasma. The study also describes levels of AOZ and AMOZ in liver of broilers treated for

7 days with either furazolidone- or furaltadone-medicated feed containing 200 mg/kg of the drugs. At day 0, levels of AMOZ and AOZ were around 5 000 and 1 000 µg/kg, declining to 50 and 20 µg/kg, respectively, after 21 days. In muscle, levels of AMOZ and AOZ were 30 and 10 µg/kg, respectively, at day 21.

McCracken et al. (2005a) performed a study with 28-day-old broilers treated via the feed for a period of 12 days, after which the animals were slaughtered and muscle and liver were collected. Feed contained 30, 100, 300, 1 000 or 3 000 µg/kg of furazolidone, furaltadone, nitrofurantoin or nitrofurazone—levels which were all well below the previous therapeutic level of 300 mg/kg. Tissues were first extracted with organic solvents prior to release of the side-chain, meaning that only protein-bound residues were taken into account. In the case of furazolidone, this resulted in AOZ levels in liver from 1.1 to about 25 µg/kg, compared with 0.3 to 10 µg/kg in muscle, with a clear linear dose–response relationship. AMOZ levels in liver and muscle at the highest dose were as high as 40 and 15 µg/kg, respectively, which is very similar to AOZ in the case of treatment with furazolidone when corrected for molecular weights. In the case of nitrofurantoin, levels of AHD in muscle were very low, whereas in liver they amounted to 35 µg/kg, which again is very similar to AOZ in the case of furazolidone treatment, expressed on a molar basis. For nitrofurazone, the situation was somewhat different, showing SEM levels in liver and muscle of up to 35 and 28 µg/kg, respectively, which on a molar basis were also much higher than observed for the other drugs.

Barbosa et al. (2011) fed 19-week-old laying hens with feed containing either 150 mg/kg of furaltadone or 100 mg/kg of nifursol for a period of 5 weeks followed by 3 weeks on clean feed. Animals were slaughtered at the end of the treatment or after the additional 3 weeks on clean feed, and muscle, liver and gizzards were examined for the parent compound and the side-chains, the latter without prior extraction of tissues. Parent compounds could be detected directly after the treatment, with levels of furaltadone in meat, liver and gizzards around 35, 40 and 55 µg/kg, and with levels of nifursol a bit lower, i.e. 15, 13 and 12 µg/kg, respectively. Parent compounds were not detectable after 3 weeks on clean feed. Levels of the AMOZ and DNSH side-chains directly after the treatment were much higher, with levels of furaltadone in meat, liver and gizzards around 1 800, 3 300 and 3 300 µg/kg, respectively, and levels of nifursol again lower, i.e. in all tissues between 300 and 350 µg/kg. Levels decreased after 3 weeks on clean feed but were still detectable, being 270, 80 and 331 µg/kg for furaltadone and 2.5, 6.4 and 10.3 µg/kg for nifursol in meat, liver and gizzards, respectively. Overall, the results are in agreement with those obtained by Zuidema et al. (2005). When similar animals were fed a diet containing a mixture of 15 mg/kg furaltadone and 10 mg/kg nifursol, roughly 10-fold lower levels of parent drugs and side-chains were detected, with some variation. This implies that, at these relatively low feed levels compared with the formerly used doses, tissue levels of AMOZ and DNSH were still well above the MRPL of 1 µg/kg at the end of the treatment period, and this was still the case after a 3-week withdrawal period for AMOZ but not for DNSH.

8.1.4.4. Horses

Despite the documented use of furaltadone in horses (Huber, 1982), no data on kinetics were identified for any of the nitrofurans.

8.1.4.5. Fish and other seafood

Heaton and Post (1968) exposed brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*) and cutthroat trout (*Oncorhynchus clarkii*) to 35 mg **furazolidone**/kg b.w. per day for 20 days, followed by a 10-day depuration period. Brown trout had the highest mean muscle residue concentration (482 µg/kg) after 10 days of medication. Mean furazolidone levels in fish fillets in all species were less than 75 µg/kg after 10 days' depuration, achieved by feeding on non-medicated feed.

Plakas et al. (1994) administered a single oral dose of 1 mg ^{14}C-furazolidone/kg b.w. (labelled in the AOZ side-chain) to channel catfish (*Ictalurus punctatus*) to examine the pharmacokinetics, tissue distribution and excretion of furazolidone. The oral bioavailability of furazolidone was 28 % in a feed

mixture. Elimination of the parent compound was rapid, with a half-life of 0.27 hours and total body clearance of 1 901 mL/kg b.w. per hour. The plasma concentration of furazolidone was highest 1 hour after dosing, and was below the LOD (< 20 µg/L) at 5 hours. Levels of furazolidone and its metabolites were highest in the excretory tissues and lowest in the muscle. Parent furazolidone comprised 10 % of the total radiolabel in muscle tissue after 8 hours and was below the LOD (< 1 µg/kg) after 24 hours. Total ^{14}C concentrations declined from 274 to 59 ng furazolidone equivalents/g between 8 and 168 hours. Bound residues accounted for 18 % of total ^{14}C in muscle after 8 hours and 33 % after 168 hours. The primary route of excretion of ^{14}C-labelled residues was via the kidney in urine and accounted for about 55 % of the oral dose.

Rainbow trout (*Oncorhynchus mykiss*) were exposed for 10 days to 135 mg/kg b.w. of ^{14}C-furazolidone, labelled in either part of the molecule, through the feed (Law and Meng, 1996). When labelled in the nitrofuran part, the amount of radiolabel was higher than in the case of furazolidone labelled in the AOZ side-chain. This suggests that part of the side-chain is cleaved either before or after formation of the protein adducts. Hepatic protein-bound radiolabel remained high for at least 30 days following dietary treatment. AOZ could be cleaved from part of the protein-bound residues, showing that AOZ is also suitable as a marker residue in trout. The effect of dose and water temperature on the amount of protein-bound ^{14}C in the liver was investigated further, showing higher bound residue formation at higher temperatures. Results indicate that protein-bound radiolabel in the muscle and liver of trout is related to the formation of reactive intermediates from furazolidone.

Guo et al. (2003) investigated the disposition of the cyano-metabolite of furazolidone, 3-(4-cyano-2-oxobutylidene amino)-2-oxazolidinone, in the orange-spotted grouper (*Epinephelus coioides*) after oral treatment with 50 mg furazolidone/kg b.w. The cyano-metabolite was mainly distributed in the serum and muscle rather than in the liver and kidney. The peak concentrations of the cyano-metabolite following oral exposure were 167.2 µg/L in serum and 283.2 µg/kg in muscle, which were reached after 5.1 and 6.7 hours, respectively. The elimination half-life of the cyano-metabolite was 4 hours.

The residues of furazolidone and its marker metabolite (AOZ) were investigated in Nile tilapia (*Oreochromis niloticus*) (Xu et al., 2006). After oral dosing with 30 mg/kg b.w. for 7 days, the maximum concentration of furazolidone in Nile tilapia was 413 µg/kg after 6 hours, whereas AOZ reached its maximum concentration (31 µg/kg) when treatment ceased. In contrast to the rapid metabolism of furazolidone, AOZ was eliminated slowly; thus, the withdrawal time in Nile tilapia was at least 22 days.

The elimination of furazolidone from Vietnamese black tiger shrimp (*Penaeus monodon*) muscle was examined for up to 28 days following 7 days' dosing (dose not given (Douny et al., 2013)). The maximum concentration of NPAOZ following 7 days' treatment was 874 ± 326 µg/kg. After the treatment ceased, residue levels decreased. However, after 28 days the concentration of NPAOZ in shrimp muscle was 115 ± 37 µg/kg.

Krongpong et al. (2008) measured the persistence of AOZ in eel (*Anguilla japonica*) following aquatic exposure to 2 or 10 mg furazolidone/L for 3 hours, with a 160-day depuration period in clean water. The half-lives of AOZ were 25.0 days in muscle and 21.6 days in liver from fish exposed to 2 mg/L furazolidone. The eels with 10 mg/L furazolidone had higher AOZ concentrations in liver and muscle following exposure than those in the low-dose group; however, the half-lives of AOZ were similar to those in fish treated with 2 mg/L furazolidone (26.6 and 21.9 days in muscle and liver, respectively).

Channel catfish were exposed via feed to 200 mg furazolidone/b.w. per day for 5 days followed by a 3-month elimination period to examine the half-life and tissue distribution of AOZ (Liu et al., 2012b[37]). Samples of muscle, skin, liver and kidney were taken from fish after1, 2, 4, 6, 8, 12, 24, 48, 96, 192, 288, 384, 480, 720, 1 080, 1 440, 2 160 and 2 880 hours; fish were not starved prior to sampling. AOZ reached peak concentration in all blood and all tissues sampled 4 hours following

[37] Original paper in Chinese. The text in this Scientific Opinion is based on the English translation of the paper.

discontinuation of the drug and the drug remained detectable in all samples examined after 1 440 hours (60 days). AOZ levels were below the LOD after 2 160 hours (90 days). The concentration of AOZ was highest in kidney tissue followed by liver tissue, skin tissue, and blood and muscle tissue. The elimination half-life of the drug in skin tissue was longest, followed by in muscle tissue, which in turn had a longer half-life than in liver and kidney tissue. The authors claimed that the study is representative of the behaviour of furazolidone in aquaculture; however, it does not appear that the dietary treatment was conducted in triplicate and there is no feed intake data presented, which are both requirements relevant for feeding trials.

Wang et al. (2010) characterised a cyano-derivative as a major metabolite of **nitrofurazone** in channel catfish. The depletion of cyano metabolite was examined in the muscle of channel catfish after single oral dosing (10 mg nitrofurazone/kg b.w.). Parent nitrofurazone was rapidly eliminated in muscle, with a half-life of 6.3 hours. The cyano metabolite was detected for up to 2 weeks, with an elimination half-life of 81 hours. The authors suggest that the cyano metabolite represents an alternative biomarker for confirming the use of nitrofurazone in channel catfish.

Chu et al. (2008) investigated the depletion of **furazolidone**, **nitrofurazone**, **furaltadone**, and **nitrofurantoin** and their tissue-bound metabolites AOZ, SEM, AMOZ and AHD, respectively, from the muscle tissue of channel catfish (*Ictalurus punctatus*) following single oral dosing with 1 mg/kg b.w. Parent compounds were quantifiable in muscle tissue within 2 hours. Peak concentrations were found after 4 hours for furazolidone (30.4 µg/kg) and after 12 hours for nitrofurazone, furaltadone and nitrofurantoin (104, 35.2 and 9.8 µg/kg, respectively). Concentrations of the parent compounds in muscle fell below the LOD (1 µg/kg) at 96 hours. Peak levels of tissue-bound AMOZ and AOZ (46.8 and 33.7 µg/kg, respectively) were measured at 12 hours, and of SEM and AHD (31.1 and 9.1 µg/kg, respectively) were measured at 24 hours. Tissue-bound metabolites were measurable for up to 56 days following treatment.

8.1.4.6. Concluding comments

The studies performed with various animal species show that, in general, the parent compounds are rapidly metabolised. Only in the case of eggs are the parent compounds detected in considerable amounts. Furazolidone has been studied most but, in general, there is limited information on metabolites of nitrofurans. Radiolabelled studies indicate the formation of a large number of different metabolites. The most studied metabolite is the open-chain cyano metabolite resulting from the six-electron reduction of the nitro-group. Studies with radiolabelled drugs also revealed the formation of protein-bound residues, both *in vivo* and *in vitro*. These adducts are likely to result from the formation of reactive intermediates during the reduction of the nitro-group. As a result, the side-chains are still intact in at least part of the bound residues and can be released by cleavage of the azomethine bond between the nitrofuran ring and the side-chain under acid conditions. Residue formation of furazolidone, furaltadone, nitrofurantoin and nitrofurazone has been studied in pigs and poultry, showing that the side-chains can be released and detected even after long withdrawal times, with some minor differences between the drugs. Initial levels after therapeutic use are in the mg/kg range but decrease to the µg/kg range after several weeks, which is thought to be the result of protein turn-over and partly the growth of animals. Nifursol was studied in broilers only, showing similar behaviours as the other nitrofurans. The side-chains can also be released from the parent compounds at low pH but the extent to which this occurs is unclear.

8.1.5. Bioavailability of bound residues

When feeding pig meat containing radiolabelled protein-bound residues of furazolidone to rats, part of the radiolabel (present in the AOZ side-chain) was detected in the urine and in the tissues of the rats, showing that these residues are bioavailable and might be of concern (Vroomen et al., 1990). This was confirmed by studies of Gottshall and Wang (1995) using bile duct-cannulated rats given meat and liver of pigs treated with radiolabelled furazolidone (label in both rings). It was concluded that 40 and 37 % of the radiolabelled compounds present in liver and muscle, respectively, of pigs slaughtered just after the last treatment were absorbed from the gastrointestinal tract. This fraction decreased to 19 %

for liver from pigs slaughtered after a withdrawal period of 45 days but remained similar (41 %) for muscle. When focusing on non-extractable radiolabelled residues only, the fraction absorbed was quite similar, being 31 and 16 % for liver of day-0 and day-45 animals, respectively, and 37 % for muscle of day-45 animals. Vroomen et al. (1990) also observed that, in the rats, part of the radiolabel was present in tissues and part was present as non-extractable, protein-bound residues. These studies show that residues in muscle and liver from treated pigs, including the non-extractable residues, are bioavailable, although the nature of the radiolabelled compounds could not be identified.

It was discussed whether the radiolabelled residues were actually adducts of the nitrofurans (drug-like) or whether the labelled nitrofuran was extensively degraded resulting in small radiolabelled fragments that were reused for the synthesis of amino acids and subsequently incorporated into proteins. The fact that AOZ could be released from part of the protein-bound residues (Hoogenboom et al., 1991c) confirmed that they were drug-like and this caused a renewed interest in the potential risk of the residues, especially regarding the fact that the AOZ side-chain could be released by acid treatment and thus potentially in the stomach. In the case of furazolidone itself, it had already been shown that the formation of AOZ in the stomach was probably the basis of one of the adverse effects (neurotoxic effects) of the drug in human patients. AOZ was shown to be an inhibitor of MAO and its inhibition would result in a decreased detoxification of compounds such as tyramine formed in the gastrointestinal tract (Stern et al., 1967). It had been hypothesised that the effect was due to the formation of 2-hydroxy-ethylhydrazine (HEH) from AOZ following ring opening, although this was never demonstrated.

In vitro studies with Caco-2 cells or perfused rat guts strongly indicated that free AOZ was released and absorbed from protein-bound residues of furazolidone (Klee et al., 1999). Free AOZ was shown to be transported from the apical to the basolateral side of either a monolayer of human Caco-2 cells or isolated perfused rat gut segments. To investigate the possible release and absorption of AOZ from bound residues, liver microsomes containing protein-bound adducts of furazolidone were first digested with hydrogen chloride and pronase and then added to either Caco-2 cells or rat gut segments. Again, AOZ was detected on the other side, suggesting release and absorption from bound residues. However, in this study, AOZ was detected after derivatisation with NBA at low pH, leaving the possibility that AOZ was not present in its free form. By applying a newly developed method for the extraction and LC-MS detection of free AOZ (Hoogenboom et al., 2002), it was shown that free AOZ was present in the blood of pigs treated orally with AOZ but also furazolidone. With the same method it was possible to show the presence of free AOZ in the blood of rats fed with pig meat containing bound residues. This demonstrated that AOZ is released during digestion and is actually absorbed.

Studies with isolated pig hepatocytes revealed that exposure to AOZ also resulted in the formation of non-extractable protein-bound residues, and that AOZ could be released from these residues by acid treatment (Hoogenboom et al., 2002). This observation also explains the results obtained by McCracken and Kennedy (1997) who fed rats with liver, kidney or muscle from furazolidone-treated pigs. It was shown that AOZ could be released from the rat tissues that were first extracted with organic solvents. This shows that AOZ is released from bound residues in the stomach of the rat and subsequently binds to proteins in the organs of the rat. In addition, bound residues in tissues from food-producing animals may be derived from both furazolidone itself and AOZ, released from the parent drug in the stomach by acid hydrolysis.

Studies on the bioavailability of bound residues or side-chains of other nitrofuran drugs are not available.

In conclusion, these studies show that protein-bound residues may be formed by metabolism of the parent compound (adduct type I), but also of the side-chains released in the stomach of animals and humans (adduct type II) (Figure 4). As shown in the case of furazolidone, the protein-bound residues are bioavailable owing to the release of the AOZ side-chain from either type of protein-bound residues in the stomach. The absorbed AOZ may subsequently be metabolised to a compound able to form adducts (adduct type II).

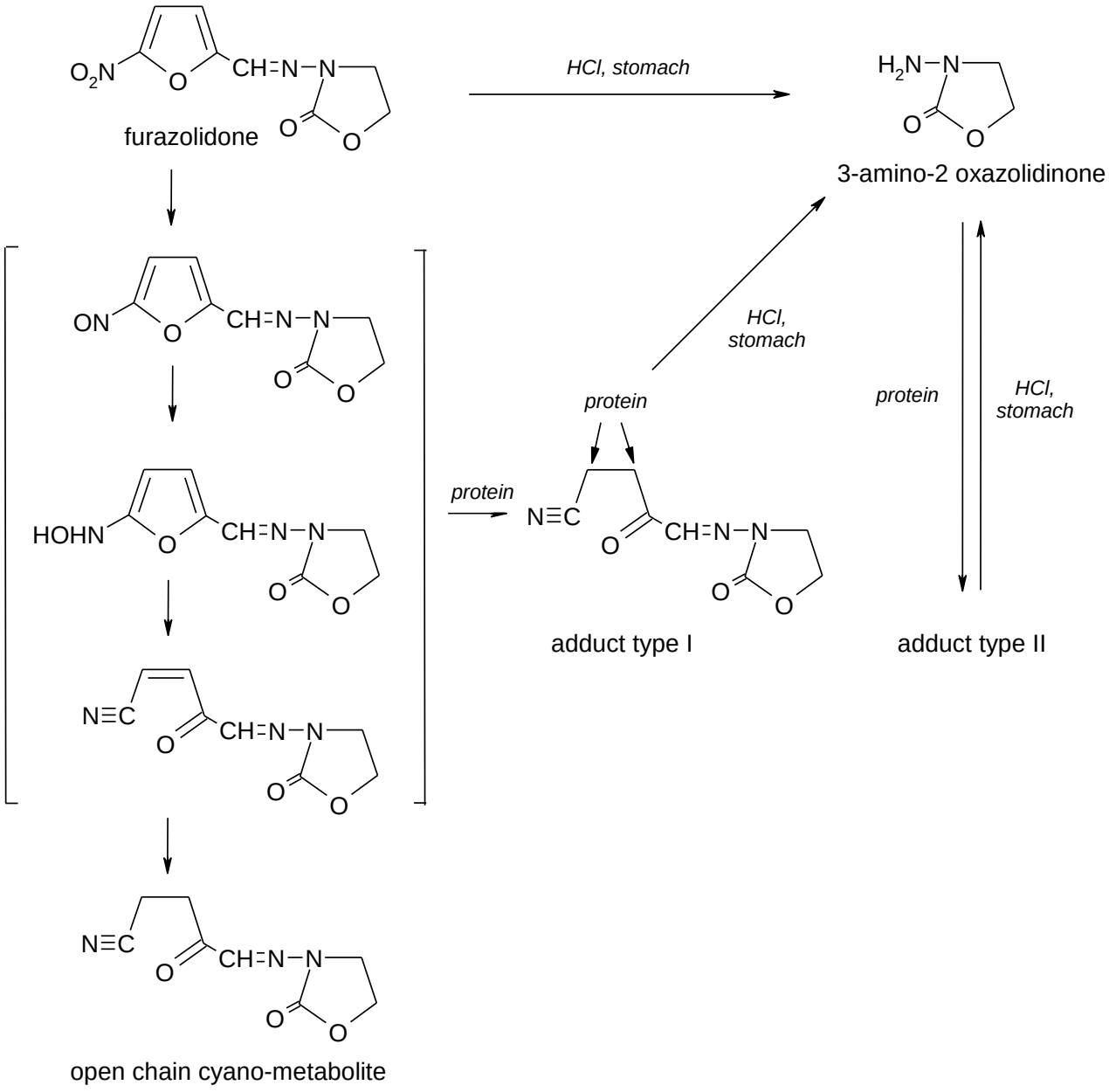


Figure 4: Biotransformation of furazolidone resulting in the formation of two types of protein-bound adducts, either from a reactive metabolite formed by reduction of the nitro-group or from AOZ released in the stomach under acid conditions from either the parent compound or protein-bound residues

8.2. Toxicity in experimental animals

8.2.1. Acute toxicity

Furazolidone has been tested for acute toxicity in rats and mice, AOZ and AMOZ in rats, nitrofurantoin in rats, mice and chickens, nitrofurazone in rats and mice, and SEM in rats and mice. Median lethal doses (LD_{50}) are reported in Appendix G, Table G.1. In summary, the oral LD_{50} value for **furazolidone** was estimated to be 1 110 mg/kg b.w. in mice and 1 508 mg/kg b.w. in rats. For **AOZ**, the oral LD_{50} value was estimated to be 2 739 mg/kg b.w. in rats. No information regarding the acute toxicity of **furaltadone** was identified. For **AMOZ**, the oral LD_{50} value was estimated to be

> 2 000 mg/kg b.w. in rats. For **nitrofurantoin**, the oral LD_{50} value was estimated to be 360 mg/kg b.w. in mice and 148 mg/kg b.w. in chicken, and ranged between 89 and 1 493 mg/kg b.w. in rats, depending on the age of the rats. No information regarding the acute toxicity of **AHD** was identified. For **nitrofurazone**, the oral LD_{50} value was estimated to be between 460 and 640 mg/kg b.w. in mice and between 590 and 800 mg/kg b.w. in rats. For **SEM**, the oral LD_{50} value was estimated to be 176 mg/kg b.w. in mice. No information regarding the acute toxicity of **nifursol** or **DNSH** was identified. The lungs are an important target organ for acute toxicity of furazolidone, nitrofurantoin and nitrofurazone in rats, mice and chickens. Decreased food consumption and body weight loss, hyperirritability, tremors, convulsions, alopecia, corneal opacity, discoloration of the lungs and decreased respiratory function were observed.

To investigate the role of oxidative stress in the toxicity of nitrofurans, several studies on antioxidants have been carried out. The acute toxic effect of **nitrofurantoin** (nitrofurantoin macrocrystals) on the lungs was tested in rats with subcutaneous (s.c.) treatment (Boyd et al., 1979). Male rats (Sprague–Dawley, 8 to 10 per group) were treated s.c. on a single occasion with 300 to 500 mg/kg b.w. nitrofurantoin in an aqueous suspension. Between 4 to 12 hours after dosing, severe toxicity in the lungs was found with respiratory suffering. Microscopic examination revealed interstitial and intra-alveolar oedema, vascular haemorrhage and consolidations (alveoli and small airways filled with dense material). The highest doses caused death owing to respiratory failure and convulsions. No pathological changes were found in organs other than the lungs. The LD_{50} for s.c. dosing with nitrofurantoin was 35 mg/kg b.w. for rats fed a vitamin E-deficient diet compared with 400 mg/kg b.w. for rats fed a normal diet. The impact of vitamin E deficiency, dietary fat and oxygen on the toxicity of nitrofurantoin was also tested. Rats (n = 10 to 15 per group) were treated for 6 weeks with feed deficient in vitamin E and with a high content of polyunsaturated fatty acids or saturated fatty acids (lard), followed by 3 weeks' treatment with a vitamin E-fortified diet (200 mg/kg diet) or a diet without vitamin E fortification. Following the pretreatments, the rats were treated s.c. with 100 mg/kg b.w. nitrofurantoin. After prefeeding with unsaturated fatty acids, all rats that were fed the diet without fortification with vitamin E before treatment died within 7 hours after nitrofurantoin treatment. Approximately 90 % died after 24 hours when pre-fed with saturated fatty acids and without a vitamin E fortification. In the groups treated with a vitamin E-fortified diet for 3 weeks before treatment with nitrofurantoin only, approximately 10 % of the rats died within 24 hours after treatment, independent of the type of fat used in pre-feeding. It was also shown that pulmonary lesions and deaths increased when rats were placed in oxygen-enriched atmospheres (sealed cages in which 2 L/min of 100 % oxygen passed) for 24 hours, directly after administration of nitrofurantoin. As indicated in the dose levels used, rats fed a vitamin E-deficient diet high in polyunsaturated fat (15, 20 and 25 mg/kg b.w. of nitrofurantoin) showed more severe pulmonary lesions, appearing at an earlier time point, than rats fed a normal diet treated with higher doses (250 or 400 mg/kg b.w.) of nitrofurantoin. Groups of rats at all dose levels were treated with or without an oxygen-enriched atmosphere for 24 hours. The rats treated in an oxygen-rich atmosphere showed much more severe lesions in the lungs than rats treated under a normal atmosphere. Rats treated at the highest doses died within 4 (400 mg/kg b.w.) and 6 (25 mg/kg b.w.) hours. Thus, the toxicity of nitrofurantoin was decreased by vitamin E but was increased by unsaturated fats in the diet and by treatment under high-oxygen atmospheres.

Effects of selenium and vitamin E deficiency on the acute oral toxicity of nitrofurantoin (nitrofurantoin macrocrystals) in chickens were studied (Peterson et al., 1982). Chickens (Leghorn, 8 days old) born from dams fed a vitamin E- and selenium-depleted basal diet for two generations were used. The LD_{50} for chickens fed the same basal diet supplemented with selenium and vitamin E was 148 mg/kg b.w. after 48 hours mortality observation. The LD_{50} for chickens fed the basal diet without supplementation was 53 mg/kg b.w. Different diets, with or without selenium and vitamin E, were also tested. It was found that selenium but not vitamin E had a protective effect against lethality from nitrofurantoin treatment in chickens. Histological examination of the lungs of treated chickens showed hyperaemia and variable oedema; some changes in the kidney were noted, but there were no signs of toxicity in the liver or the heart.

The effect of nitrofurantoin (chemical form not specified) on blood pressure was investigated in rats (Murakami et al., 1989). Wistar rats (250 g, n = 7) were treated with nitrofurantoin at single i.v. doses of 0.1 to 0.3 mg/rat, corresponding to 0.4 and 1.2 mg/kg b.w., respectively, for a 250 g rat. The enzyme activity of GSH reductase decreased in the brain stem and the hypothalamus, which led to an increased ratio of oxidised GSSG to reduced glutathione. In addition, blood pressure and heart rate, as well as plasma renin activity and plasma noradrenaline, increased in rats. These effects were blocked or reduced by the sympathetic ganglion blocker pentolinium tartrate. It was concluded that the decreased activity of GSH reductase caused by nitrofurantoin treatment led to increased blood pressure by activating the sympathetic nervous system and the renin–angiotensin system.

8.2.2. Repeated-dose toxicity

No repeated-dose toxicity studies were identified for **furazolidone**.

AOZ, the marker metabolite for furazolidone, was tested in repeated-dose toxicity studies in rats and dogs.

AOZ (crystalline, suspended in water and included in pellets) was fed to rats (Wistar, 9 weeks old, five of each sex in each dose group) in the diet for 14 days in concentrations of 0, 100, 500, 2 500 or 10 000 mg/kg feed (NOTOX, 1995a). These concentrations correspond to 0, 12, 60, 300 and 1 200 mg/kg b.w. per day, respectively, using the default factor of 0.12 as recommended by the EFSA SC (2012). No mortality was found during the study, but animals in the two highest dose groups died after blood sampling just before sacrifice. Dose-related clinical signs such as piloerection, hunched postures, rales (abnormal lung sounds) and pale appearance were found in the three highest dose groups, and laboured respiration was found in the highest dose group. No clinical signs were found in the 12 mg/kg b.w. per day group or in the control group. Food consumption, body weight and body weight gain dose-dependently decreased in all treated groups compared with the control group. Haematology analyses showed that there was damage to the red blood cells in all treated groups and the total leucocyte counts were very low in the three highest dose groups. Furthermore, increased serum bilirubin, aspartate aminotransferase (AST) levels and alkaline phosphatase (ALP) levels were found in the two highest dose groups. Decreased protein levels were found in all treated males, but in females were found in only the two highest dose groups. Relative weights of spleen, kidney and liver increased in the two highest dose groups. Histopathology revealed congestion, haemosiderosis and increased haematopoiesis in the spleen of all treated animals. In the groups treated with 60 mg/kg b.w. per day and higher, effects on the thymus, intracellular oedema in tubules of the kidney and erosion of the fundic mucosa were found. In the two highest dose groups, vacuolisation in the liver was noted and, in the highest dose group, reduced spermatogenesis was also found. The increased levels of the enzymes AST and ALP, the increased liver weights and the macroscopic changes indicate that AOZ caused toxic effects in the liver. Effects in the lungs were not investigated, but laboured respiration was found in the 1 200 mg/kg b.w. per day dose group and rales were seen in dose groups at or higher than 60 mg/kg b.w. per day. According to the authors, effects on the spleen, red blood cell parameters and weight gain were found in the lowest dose group (12 mg/kg b.w. per day). However, because no numerical data were provided, the CONTAM Panel was not able to verify this conclusion.

AOZ (crystalline, suspended in water and included in pellets) was fed to rats (Wistar, 9 weeks old, 10 of each sex in each dose group) in the diet for 90 days in concentrations of 0, 10, 50 or 100 mg/kg feed (NOTOX, 1995b). These concentrations correspond to 0, 0.9, 4.5 or 9 mg/kg b.w. per day, respectively, using the default factor of 0.09 as recommended by the EFSA SC (2012). No deaths or relevant clinical signs were found during the treatment period. Body weights and body weight gain and food consumption decreased in animals treated with 4.5 or 9 mg/kg b.w. per day. No effects on body weight, body weight gain or feed consumption were found in the lowest dose group. Red blood cell parameters decreased in females and males treated with 9 mg/kg b.w. per day, but in only males treated with 4.5 mg/kg b.w. per day. Upon histological examination, the only finding was an increased severity of diffuse hepatocellular vacuolisation in the highest dose group, but no effects on the lungs were identified. According to the authors, no changes in haematology parameters, clinical chemistry

analyses or organ weights, or histological examination were found in the lowest dose group, 0.9 mg/kg b.w. per day, compared with control animals. However, because no numerical data were provided, the CONTAM Panel was not able to conclude on a NOAEL from this study.

Dogs (Beagle dogs, 3–4 months old, three of each sex in each dose group) were treated orally with AOZ (crystalline powder, suspended in water) in the diet for 90 days with 0, 1, 3 or 6 mg/kg b.w. per day (Brinck et al., 1995). No treatment-related mortality or clinical signs were found during the study. Effects on body weight, body weight gain or food consumption were not statistically significantly different from the control group. Increased relative liver weights were found in males and increased absolute and relative spleen weights were found in females in the highest dose group. Several haematology parameters were dose-dependently changed (decreased or increased) in treated groups compared with controls, showing that AOZ caused a macrocytic, hypochromic anaemia. In animals in the two highest dose groups, the thrombin time was prolonged but the fibrinogen levels were normal, which causes inhibition of fibrin formation. Clinical chemistry analyses of serum showed significant increased bilirubin, ALP and gamma-glutamyl transferase (GGT), as well as increased AST, alanine aminotransferase (ALT) and triglyceride levels, in the two highest dose groups indicating a dose-dependent cholestasis. In the lowest dose group, only ALP in males and GGT in females significantly increased. Many organs were histopathologically examined, including the lungs. Macroscopic changes in the liver were found in only the highest dose group. Perivascular cell accumulations in the liver were found in all treated groups. An increased cellularity in the bone marrow, femur and sternum was found in all treated females and in all males treated with 3 and 6 mg/kg b.w. per day, but in only one male in the lowest dose group. Congestions in the spleen were found in all treated animals and in one out of six control animals. AOZ caused liver toxicity and anaemia at all dose levels. Therefore, a NOAEL in dogs cannot be identified from this study.

No repeated-dose toxicity studies were identified for **furaltadone and AMOZ**.

Toxicity of **nitrofurantoin** was tested for 14 days (dose range finding studies) and 13 weeks in mice and rats (NTP, 1989).

Nitrofurantoin (microcrystalline powder) was fed to mice (B6C3F1, 4–6 weeks old, five of each sex per group) for 14 days at concentrations of 0, 600, 1 300, 2 500, 5 000 or 10 000 mg/kg feed (NTP, 1989). These concentrations correspond to 0, 120, 260, 500, 1 000 or 2 000 mg/kg b.w. per day, respectively, using the default factor of 0.2 as recommended by the EFSA SC (2012). Four out of five males and females in the 2 000 mg/kg b.w. per day dose group and one out of five in the 1 000 mg/kg b.w. per day dose group died between days 6 and 14. All mice in the 2 000 mg/kg b.w. per day dose group and male mice in the 1 000 mg/kg b.w. per day dose group lost weight, but the weights in the lower dose groups (120, 260 and 500 mg/kg b.w. per day) were not different from the controls. Clinical signs in mice from the highest dose group were lethargy, sunken eyes and altered gait. No histological examination was conducted.

Nitrofurantoin (microcrystalline powder) was fed to mice (B6C3F1, 5–6 weeks old, 10 of each sex in each group) for 13 weeks at concentrations of 0, 300, 600, 1 300, 2 500 or 5 000 mg/kg feed (NTP, 1989). These concentrations correspond to 0, 60, 120, 260, 500 or 1 000 mg/kg b. w. per day, respectively, using the default factor of 0.2 as recommended by the EFSA SC (2012). Most mice survived except 2 out of 10 in the highest dose group and 1 out of 10 in the lowest dose group. Clinical signs were lethargy, hypothermia and sunken eyes in the highest dose group. The body weight gain decreased in the highest dose group to approximately 85 % of that of the control group. Feed consumption was not affected by treatment. The relative liver weights decreased in the two lowest dose groups, but not in the higher dose groups. Upon histopathological examination, minimal to mild necrosis of the kidney epithelium was observed in two out of nine high-dose males, necrosis of the ovarian follicle was observed in 8 out of 10 high-dose females and minimal to mild degeneration of the germinal epithelium of the testes, accompanied by aspermatogenesis, was observed in male mice administered a dose of 260 mg/kg b.w. per day or higher. No such effect was observed in the lower dose groups. A NOAEL of 120 mg/kg b.w. per day was identified from this study.

Nitrofurantoin (microcrystalline powder) was fed to rats (F344/N, 4–5 weeks old, five of each sex in each group) for 14 days at concentrations of 0, 1 300, 2 500, 5 000, 10 000 or 20 000 mg/kg feed (NTP, 1989). These concentrations correspond to 0, 156, 300, 600, 12 00 or 2 400 mg/kg b.w. per day, respectively, using the default factor of 0.12 as recommended by the EFSA SC (2012). All rats survived, but dose-related decreases in weight gain were observed at doses ≥ 300 mg/kg b.w. per day, reaching 47 % of that of the control group for males and 55 % for females in the highest dose group at the end of the study. Rats from the four highest dose groups had lower feed intakes than control animals during the first week of treatment, but, during the second week, the feed intake increased so that it was close to the control group and the lowest dose group. Rough hair coat, lethargy, sunken eyes and brighter yellow urine was found as clinical symptoms in all dose groups. Necropsy, but not histological examination, was performed. In the highest dose group, a discoloration to blue was seen in the joints.Nitrofurantoin (microcrystalline powder) was fed to rats (F344/N, 5–6 weeks old, 10 of each sex in each group) for 13 weeks at concentrations of 0, 600, 1 300, 2 500, 5 000 or 10 000 mg/kg feed (NTP, 1989). These concentrations correspond to 0, 54, 117, 225, 450 or 900 mg/kg b.w. per day, respectively, using the default factor of 0.09 as recommended by the EFSA SC (2012). Only 1 out of 10 females died during the study. Weight gain decreased dose dependently at doses ≥ 225 mg/kg b.w. per day, reaching 47 % of that of the control group for males and 41 % for females in the highest dose group at the end of the study. Feed consumption was not influenced. Upon histopathological examination, aspermatogenesis was found, as well as minimal to mild degeneration of the germinal epithelium of the seminiferous tubules of the testes, in 29 out of 30 male rats treated with doses ≥ 225 mg/kg b.w. per day. A dose-dependent necrosis of the ovarian follicles in females was found in groups treated with 900 mg/kg b.w. per day (8 out of 10), 450 mg/kg b.w. per day (3 out of 10) and 225 mg/kg b.w. per day (1 out of 10). No effects were observed in the lungs. In addition, the relative liver weight was significantly higher in the two highest dose groups. From this study, a NOAEL of 117 mg/kg b.w. per day in rats treated for 13 weeks with nitrofurantoin in feed was identified.Rabbits (New Zealand White, 12 in each group and six in each control group) were treated with nitrofurantoin (chemical form not specified, 0.1 mg/mL aqueous solution) at a dose of 6 mg/kg b.w. per day for 1 week or 3, 6 or 12 months through drinking water to study the effect on the bladder (Levin et al., 1988). Up to 12 months' treatment did not affect bacterial adherence to the rabbit bladder and histopathology of the bladders did not show any harmful effect in the epithelial layer, mucosal layer or mucin coating.

No repeated-dose toxicity studies were identified for **AHD**.Toxicity of **nitrofurazone** (chemical form not specified) was tested for 14 days (dose range finding studies) and for 13 weeks in both mice and rats (NTP, 1988).

Mice (B6C3F1, 6–8 weeks old, five of each sex in each group) were fed nitrofurazone for 14 days at concentrations of 0, 630, 1 250, 2 500, 5 000 or 10 000 mg/kg feed, corresponding to 0, 126, 250, 500, 1 000 or 2 000 mg/kg b.w. per day, respectively, using the default factor of 0.2 as recommended by the EFSA SC (2012). All mice in the 500, 1 000 and 2 000 mg/kg b.w. per day groups and three out of five males in the 250 mg/kg b.w. per day group died before the end of the study. Feed consumption in groups treated with doses higher than 126 mg/kg b.w. per day was dose-dependently decreased. Treated mice had rough hair coats and convulsive seizures (NTP, 1988).

Mice (B6C3F1, 5 weeks old, 10 of each sex in each group) were treated with nitrofurazone for 13 weeks at concentrations of 0, 70, 150, 310, 620 or 1 250 mg/kg feed (NTP, 1988). These concentrations correspond to 0, 14, 30, 62, 124 or 250 mg/kg b.w. per day, respectively, using the default factor of 0.2 as recommended by the EFSA SC (2012). Mortality in males and females increased in the two highest dose groups; only 1out of 10 females and 4 out of 10 males survived during the 13 weeks. Final weights decreased in only the highest dose group. Relative liver weights increased by 35 % in the two highest dose groups compared with the control group. Liver weights decreased in the two lowest dose groups, which resulted in a 20 % decrease in relative liver weight compared with the control group. No effects in the lungs were found. Convulsive seizures and hyperexcitability were noted in both sexes at doses at or higher than 124 mg/kg b.w. per day. Moderate and severe testicular hypoplasia were found in the two highest dose groups: in 8 out of

10 mice in the 250 mg/kg b.w. per day dose group and 9 out of 10 mice in the 124 mg/kg b.w. per day dose group. From this study, the CONTAM Panel identified a NOAEL of 62 mg/kg b.w. per day for mice treated orally with nitrofurazone for 13 weeks.

Nitrofurazone was fed to rats (F344/N, 6–7 weeks old, five of each sex in each group) for 14 days at concentrations of 0, 630, 1 250, 2 500, 5 000 or 10 000 mg/kg feed. These doses correspond to 0, 75.6, 150, 300, 600 or 1 200 mg/kg b.w. per day, respectively, using the default factor of 0.12 as recommended by the EFSA SC (2012). Mortality in rats increased at the two highest dose levels. In all rats, rough hair coats and lethargy were found. Rats treated with 300 mg/kg b.w. per day and higher suffered from intermittent seizures and lethargy. A dose-dependent reduction in weight gain was found in all dose groups compared with controls. Food intake decreased in both males and females at doses above 75.6 mg/kg b.w. per day. Histopathology was performed in 10 % of the animals, indicating that males from all dose groups failed to produce sperm cells. No effects on the lungs were recorded.Rats (F344/N, 4–6 weeks old, 10 of each sex in each group) were treated with nitrofurazone for 13 weeks at doses of 0, 150, 310, 620, 1 250 or 2 500 mg/kg feed (NTP, 1988). These doses correspond to 0, 13.5, 28, 56, 112.5 or 225 mg/kg b. w. per day, respectively, using the default factor of 0.09 as recommended by the EFSA SC (2012). No mortality was found, but body weight gain at the end of the study was dose-dependently decreased at doses of 28 mg/kg b.w. per day in males and 56 mg/kg b.w. per day in females and higher. Feed consumption decreased in the highest dose group. Relative liver weights were significantly higher in all treated animals (males and females), but without any dose–response relationship. Stimulus-induced convulsive seizures were observed in the highest dose group, and hyperexcitability was found in females in the two highest dose groups and in males at only the highest dose. No effects in the lungs were found. Osteoporosis was found in males from 56 mg/kg b.w. per day and higher, and in females in the two highest dose groups. Nitrofurazone caused moderate to severe degeneration of the seminiferous epithelium in the testes in all males treated with doses of 28 mg/kg b.w. per day and higher. From this study, a NOAEL of 13.5 mg/kg b.w. per day could be identified.Rats were given nitrofurazone (macrocrystals) in the diet at concentrations of 1 000, 2 000 or 4 000 mg/kg for 5 weeks (Krantz and Evans, 1945), corresponding to 90, 180 or 360 mg/kg b.w. per day, respectively, using the default factor of 0.09 as recommended by the EFSA SC (2012). The rats in the highest dose group showed cachexia and hyperexcitability, and died during the first week. Histopathology of these rats (n = 4) showed small focal cellular and necrotic areas in the liver and coagulated albuminous fluid in the tubules in the kidney. Rats fed 90 or 180 mg/kg b.w. per day showed no effects on growth rate but higher white blood cell counts. Mild histopathology changes such as cloudy swelling of the liver cells and of the tubules in the kidney were found in some animals from the groups treated with 90 and 180 mg/kg b.w. per day. No histopathology of the lungs was conducted. The study is poorly reported.

Nitrofurazone (solid crystals, ground and included in the diet) was fed to rats (Wistar strain, 15 males and seven females) for 4 to 15 weeks at concentrations of 2 000 or 3 000 mg/kg feed, corresponding to 180 or 270 mg/kg b.w. per day, respectively, using the default factor of 0.09 as recommended by the EFSA SC (2012) (Miyaji, 1971). In this study, furylfuramide and sorbic acid were also tested. A markedly decreased body weight was found in the rats treated with 270 mg/kg b.w. per day of nitrofurazone for 4 weeks, but when treated with 180 mg/kg b.w. per day the decrease was less. Therefore, the dose of 180 mg/kg b.w. per day was chosen when rats were treated for 1 to 15 weeks. Relative liver weight increased in the nitrofurazone-treated group. A distinct atrophy of the testes was found in rats treated for more than 1 week. Histopathological examination after 4 weeks of treatment showed enlarged liver cells. After 10 weeks of treatment, hypertrophic liver cells became visible, but this effect was reversible. Histochemistry on the liver showed no changes in ALP, phosphorylase or glucose-6-phosphatase activity.

Rats (male Fischer, 10–11 weeks old) were treated with nitrofurazone (chemical form not specified, suspension in 0.5 % methylcellulose) orally at a dose of 80 mg/kg b.w. per day either as a single dose or as repeated doses for 1, 2, 3, 5 or 7 days (Ito et al., 2002). The body weight of the rats treated with 80 mg/kg b.w. per day was unchanged after dosing for 3 consecutive days, but decreased slightly after dosing for 5 or 7 days. Incorporation of bromodeoxyuridine (BrdU) in hepatocytes, indicating

increases in proliferating cells, increased reversibly after 1 and 2 days' dosing with 80 mg/kg b.w. per day, but decreased somewhat after 3 days' dosing, despite maintenance of treatment. Enzymes in plasma were not affected by treatment with nitrofurazone (80 mg/kg b.w. per day) for 1 to 3 consecutive days. The relative liver weight gradually increased during dosing up to 3 days with 80 mg/kg b.w. per day and remained at the same level thereafter. Histopathology, carried out on the liver from rats treated with two doses of 80 mg/kg b.w. per day only, revealed no cell damage but increased amounts of tangled, dark stained threads of chromosomes, i.e. mitotic figures. Mitotic figures can indicate an abnormal mitosis but, in this study, the effect was only minor. In addition, rats were treated with 20, 40 and 80 mg/kg b.w. per day for 2 days to study the BrdU incorporation in hepatocytes, for which a dose-dependent increase was observed. The authors concluded that nitrofurazone caused reversible hepatocyte proliferation without loss of cells.

Nitrofurazone was tested in monkeys (*Macacus rhesus*, n = 2) treated orally with 300 mg nitrofurazone (macrocrystals) daily for 5 weeks (Krantz and Evans, 1945). No effects on body weight, electrocardiogram, electroencephalogram or haematology parameters were found. Histopathology examination in liver and kidney showed slight changes in liver but not in kidney. An additional three monkeys were treated with 500 mg nitrofurazone for 6 days. Histopathology analysis showed minor changes such as pale, granular and swollen liver cells, but no changes in the kidney. The study is poorly reported.

Juvenile rats (Sprague–Dawley) were treated orally by gavage for 28 days with **SEM** hydrochloride dissolved in water (Maranghi et al., 2009). The rats were treated from postnatal day (PND) 23 until PND 51 with 0, 40, 75 or 140 mg/kg b.w. per day (n = 10/group). The concentrations in the drinking water were adjusted every 2 days as body weight changed with time. All animals were sacrificed at PND 60 and histological examination was carried out in various organs—thyroid, adrenals, thymus, spleen, uterus, ovaries and testes—and in the coxal–femoral joints. Significant mortality was found in the two highest dose groups, 19 and 20 %, compared with 0 % in the control group. Body weight gain was dose-dependently decreased during treatment in all males but in only the highest dose group in females. However, during the recovery period (PND 51 to 60) the weight gain was comparable to the control group in males and females. Food consumption decreased in all animals in the highest dose group but in only females in the mid-dose group. Histology showed an absence of mineralisation in the epiphyseal cartilage in all male animals treated with SEM, but not in the control group. In females, no mineralisation was found in one out of seven individuals in the control group and in six out of seven, four out of five and five out of seven rats in the 40, 75 and 140 mg/kg b.w. per day dose groups, respectively. In the thymus and thyroid, alterations in the histology were found in the two highest dose groups. In the high- and mid-dose groups, the ratio of the area of endometrium/myometrium of the uterus decreased. A dose-related significant increase in primary and secondary oocytes with condensed chromatin was found in the two highest dose groups, and a reduced number of corpora lutea was found in the highest dose group. Alteration in the adrenals was found in only females in the mid- and high- dose groups. Increased haematopoieses and the presence of megakaryocytes were found in animals in the highest dose group. Absence of mineralisation in the epiphyseal cartilage in males was found at all dose levels. The NOAEL for toxic effects of SEM in juvenile rats would therefore be lower than 40 mg/kg b.w. per day.

In a similar experiment (same protocol, same dose levels) Maranghi et al. (2010) studied, in particular, the effects of SEM on endocrine homeostasis. As in the previous study (see above) a dose-dependent decrease in body weight gain was observed in all treated males (no numerical data reported). Increased mortality (20 % compared with the control) was observed in male and female rats at 75 and 140 mg/kg b.w. per day. Food consumption decreased in high-dose males and in females at 75 and 140 mg/kg b.w. per day. Female rats showed a dose-related delayed timing of vaginal opening, which was significant in the high-dose group only. In male rats, the timing of preputial separation was shortened at 75 and 140 mg/kg b.w. per day, and delayed at 140 mg/kg b.w. per day. A dose-dependent decrease in serum levels of 17β-estradiol (E2), which was significant in the two highest dose groups, was observed in female rats at the end of the experiment (PND 62). In males, the level of dihydrotestosterone decreased at all dose levels, but without a clear dose–response relationship. In

hepatic microsomes, aromatase activity significantly increased in high-dose males and in females at 75 and 140 mg/kg b.w. per day, although, in females, no clear dose–response relationship was found. The authors concluded that SEM administration to peripubertal rats appeared to act as an endocrine disrupter in both sexes, resulting in the alteration of the onset of puberty and sex steroid serum levels (see also Section 8.3). No clear NOAEL could be derived from this study.

SEM hydrochloride was tested in rats (Wistar Hannover, 6 weeks old) treated with 0, 250, 500 or 1 000 mg/kg feed for 90 days, with 10 males and 10 females in each group (Takahashi et al., 2009). These doses correspond to 0, 23, 45 or 90 mg/kg b.w. per day, respectively, using the default factor of 0.09 as recommended by the EFSA SC (2012). No deaths occurred during treatment. Some rats showed posture and gait abnormalities. Body weight gain was lower in the highest dose group than in controls in males from week 1 and in females from week 4. Haematology analysis showed decreased segmented neutrophils and increased lymphocytes in both females and males in the highest dose group. Some clinical chemistry values were changed in a treatment-related manner. The ratio of albumin–globulin and total bilirubin increased in the high-dose males and elevated levels of creatinine and ALT were found in males in the 45 and 90 mg/kg b.w. per day dose groups. Inorganic phosphate increased in females from the 45 and 90 mg/kg b.w. per day dose groups and ALT and ALP increased in females from the highest dose group. In both males and females, body weights decreased in the highest dose group. Several relative organ weights increased in the highest dose group, e.g. brain, spleen, adrenals, kidneys and testes in males, and brain, heart and kidneys in females. The relative weights of thymus, heart, liver and lungs decreased in males in the 90 mg/kg b.w. per day dose groups. Clinical signs were enlargement and deformation of the knee joint in all rats treated with 45 and 90 mg/kg b.w. per day and in 1 out of 10 rats in the 23 mg/kg b.w. per day dose group. The same effects were found in the wrist joints in the highest dose group. A prominence of the thorax was found in the two highest dose groups in both females and males. In males from all dose groups, a stiff flexion of the tail was found. Histological examination of bones showed a dose-dependent deformation of limbs and osteochondral lesions. Disarrangements, fissures, increased connective tissues, thickening of bones and deformations of the cartilage were found in both sexes, but were more severe in male rats. Dose-dependent changes were also found in the thoracic aorta, in which interlaminar spaces had a rod or globular appearance in treated groups compared with a fibrillar form in the control group. Toxic effects were found in all dose groups and no NOAEL could be identified in this study.

Nifursol (yellow powder as a 10 % premix in lactose) was given to rats (Sprague–Dawley, 10 of each sex in each group) through feed at concentrations of 0, 200, 400, 600, 800 or 1 000 mg/kg for 13 weeks (Wood et al., 1984). The concentrations in feed correspond, respectively, to reported average doses of 0, 13.7, 28.0, 39.7, 53.6 or 67.2 mg/kg b.w. per day for males, and to 0, 14.9, 30.2, 44.0, 61.8 or 78.8 mg/kg b.w. per day for females. Ten additional females and males treated with the highest dose for 13 weeks were kept for a 4-week recovery period receiving control feed. Clinical signs observed comprised yellow staining of the beds and of the fur of treated animals, which was considered to be caused by the colour of the substance. Body weight gain decreased in males and females in the highest dose group. Food intake in the three highest dose groups was lower in males, but not in females. Both food intake and weight gain increased slightly during the 4-week recovery. Haematology tests showed that red blood cell parameters were significantly influenced by treatment. Haemoglobin levels were slightly lower than the controls in females treated with 61.8 mg/kg b.w. per day or higher for 13 weeks. Erythrocytes increased in high-dose males after 6 and 13 weeks but decreased in females treated with 30.2 mg/kg b.w. per day and higher after 6 weeks and with 44.0 mg/kg b.w. per day and higher after 13 weeks. Mean corpuscular haemoglobin concentration (MCHC) was slightly increased in males treated with 28 mg/kg b.w. per day or higher after 6 weeks and in all males after 13 weeks, but there was no clear dose–response relationship. MCHC was also increased in females in the two highest groups after 6 weeks and in females treated with 30.2 mg/kg b.w. per day or more after 13 weeks. The mean corpuscular volume (MCV) decreased in males treated with 28.0 mg/kg b.w. per day or higher for 6 weeks and in both males and females treated with 61.8 mg/kg b.w. per day or more for 13 weeks. After 6 weeks packed cell volume (PCV) was slightly decreased in males at all dose levels, but without a clear dose-response relationship, and in females from 30.2 mg/kg b.w. per day onwards. At the end of the study (week 13) PCV was significantly and

dose- relatedly decreased in females at a dose of 30.2 mg/kg b.w. per day or higher, and in males in the two highest dose groups. No significant findings were noted after the 4-week recovery period, for either red blood cell parameters or lymphocytes. Clinical chemistry analysis showed that globulin increased in all females, but without a clear dose–response relationship, in week 6 or 13, and in males in the two highest dose groups in week 6 only. No treatment-related macroscopic findings were identified. Spleen weight increased in females and males at all dose levels but without a clear dose–response relationship. After recovery, the spleen weights were close to the weights in the control group. Based on effects on red blood cell parameters (particularly on MCV in males and PCV in females at week 13), a NOAEL of about 14 mg/kg b.w. per day was identified for effects in rats treated with nifursol for 13 weeks.

No repeated-dose toxicity studies were identified for **DNSH**.

Conclusions

No repeated-dose toxicity studies were identified for **furazolidone**.

AOZ was tested in a 90-day study with rats and in a 90-day study with dogs. Hepatotoxicity, decreased body weight gain and anaemia were observed at the lowest tested dose of 1.2 mg/kg b.w. per day in rats and at 1 mg/kg b.w. per day in dogs.

No repeated-dose toxicity studies were identified for **furaltadone** and **AMOZ**.

In studies on repeated-dose toxicity of **nitrofurantoin** in rats, mice and rabbits, the main toxic effects of nitrofurantoin were on liver, kidney, testes (resulting in aspermatogenesis) and necrosis of the ovarian follicles. In a 13-week rat study, a NOAEL of 117 mg/kg b.w. per day was identified, based on effects on the testes in males and on ovarian follicles in females. In a 13-week mice study, a NOAEL of 120 mg/kg b.w. per day was identified based on effects on the testes.

No repeated-dose toxicity studies were identified for **AHD**.

Nitrofurazone was tested in rats, mice, monkeys and dogs. Nitrofurazone caused the same effects as nitrofurantoin, with the exception of necrosis of the ovarian follicles in female rats and mice. The lungs were investigated in some studies (e.g. 13-week studies with nitrofurantoin and nitrofurazone in mice and rats) but no effects were reported. The lowest doses of nitrofurazone that did not cause effects in mice and rats were 62 and 13.5 mg/kg b.w. per day, respectively. Therefore, a NOAEL of 13.5 mg/kg b.w. per day was established for effects on the testes in rats.

In a 28-days study, **SEM** caused absence of mineralisation in the epiphyseal cartilage of juvenile rats at the lowest tested dose of 40 mg/kg b.w. per day. Therefore, no NOAEL could be identified. In a 90-day study in rats, severe deformation of limbs, osteochondral lesions, altered form of the interlaminar spaces in the thoracic aorta and decreased body weight gain were observed. Toxic effects were found in all dose groups, including the lowest dose tested of 23 mg/kg b.w. per day.

In rats, **nifursol** caused slight changes in red blood cell parameters. From this 13-week study, a NOAEL of about 14 mg/kg b.w. per day was identified.

No repeated-dose toxicity studies were identified for **DNSH**.

8.2.3. Immunotoxicity

No description of *in vivo* immunotoxicity studies or effects were identified in the literature. One study reported inhibition *in vitro* by various nitrofurans of mitogenesis in stimulated human peripheral T-lymphocytes. This was seen at levels above 1–4 µg/mL, together with slight to moderate cytotoxicity (based on cell survival) (Mercado et al., 1991). The specificity as regards the immune system and the relevance for the *in vivo* situation (including dose–response relationship) cannot be assessed. It should

be noted that instances of autoimmune effects associated with liver toxicity caused by nitrofurantoin have been reported in humans (see Section 8.4.1).

8.2.4. Developmental and reproductive toxicity

8.2.4.1. Studies on spermatogenesis

Miyaji et al. (1964) treated rats (Donryu strain, 6 weeks old, n = 3) by gavage with 42 different nitrofuran compounds at 100 mg/kg b.w. per day for 7 days, testing for testes toxicity. **Nitrofurazone** (chemical form not specified) caused more severe toxicity than **furazolidone** or **furaltadone**, shown by histopathological changes in testes and reduced relative weights of testes. Testicular lesions were also found in rats treated with nitrofurazone at doses of 0.2 % in the diet for 9 days, corresponding to 240 mg/kg b.w. per day, using the default factor of 0.12 as recommended by the EFSA SC (2012). Relative testes weights decreased with time of treatment to about 45 % of the controls after 9 days. Atrophy and degeneration of the seminiferous tubules, with loss of spermatozoa, were found during the 9-day treatment, but no effects on the Leydig cells were noticed.

The effect of **furazolidone** (suspension in sodium carboxymethylcellulose) on testes, epididymis and selected nuclei of the hypothalamus was tested in groups of rats (Wistar, n = 7) treated with 0, 50 or 200 mg/kg b.w. per day by gavage for 5 days (Zimmermann et al., 1993). Testes and epididymis were analysed by light microscopy and the hypothalamus was analysed by morphometric methods. No changes in testes and epididymis were found in the low-dose group, but in the high-dose group clear signs of atrophy of the testes together with decreased weights of the testes and epididymis were found. The seminiferous tubules were clearly decreased and nearly collapsed. The epithelium of the tubules was damaged, which meant that early spermatids were absent. Morphometric analysis of the hypothalamus showed a dose-dependent increased volume of the nucleus of the hypothalamus connected to sexual centres.

No studies on spermatogenesis for **AOZ** were identified.

For **furaltadone**, only one study on spermatogenesis was identified (see above).

No studies on spermatogenesis for **AMOZ** were identified.

The effect of **nitrofurantoin** (chemical form not specified) on testes function was tested in white rats (strain not reported, n = 36 for 10 and 85 mg/kg b.w. per day dose groups and n = 18 for control group) treated by gavage for 1 month (Yunda et al., 1974). Rats were sacrificed 2, 20 or 48 days after the last treatment with nitrofurantoin and then testes and epididymis were removed and analysed. Spermatozoa were prepared and analysed. The spermatogenetic index decreased dose dependently. The number of tubuli containing spermatozoa, and the concentration and motility of spermatozoa, decreased dose dependently. In the highest dose group, the percentages of dead and pathological spermatozoa increased. Regeneration of spermatogenesis was not reached until 48 days after treatment, as there were still tubuli containing detached spermatogenic epithelium. It was also shown that cysteine and vitamins C, B1, B6 and niacin, could prevent the toxic effect on spermatogenesis caused by the highest dose of nitrofurantoin. Nitrofurantoin caused a clear toxic effect on spermatogenesis in rats at a dose of 10 mg/kg b.w. per day and higher.

No studies on spermatogenesis for **AHD** were identified.

Singh and Chakravarty (2001) treated male mice (strain Parkes, 12–14 weeks old, five per group) with **nitrofurazone** (chemical form not specified) by gavage at 64 mg/kg b.w. per day. The animals were treated either for 10 days and sacrificed 24 hours after the last administration or for 20 days and sacrificed 24 hours or 56 days after the last administration. One untreated group and one group treated with distilled water were included as controls. Body weights and weight of the epididymis were not affected by treatment. Weights of testes and seminal vesicles decreased in mice sacrificed 24 hours after the last treatment, and sperm analysis revealed a decreased motility and decreased number of

spermatozoa in cauda epididymis. These effects were more severe after 20 days than after 10 days of treatment. Histology of mice sacrificed 24 hours after the last administration showed a regressive change in the seminiferous tubules of the testes. Again, this effect was more severe after longer treatment (20 versus 10 days). Multinucleated giant cells were found in the seminiferous tubules of treated rats, indicating germ cell degeneration. In mice sacrificed 56 days after the last administration, the weights of testes and seminal vesicles, sperm analysis and histology did not differ from the control groups. Thus, in this study, reversible alterations in male reproductive organs were found in mice treated orally with 64 mg/kg b.w. per day for 10 or 20 days.

Testicular toxicity was studied in rats (male, Sprague–Dawley, 5 weeks old, 10 rats in each dose group) treated with nitrofurazone (chemical form not specified) by gavage at doses of 50 mg/kg b.w. per day for 2 or 4 weeks or 100 mg/kg b.w. per day for 2 weeks (Ito et al., 2000). Control animals were dosed with 0.5 % methylcellulose for 4 weeks. Clinical signs in the high-dose group were salivation, decreased locomotor activity and a prone position, and three animals died on day 8 to 14. In the low-dose group, only one animal died and the others did not show any clinical signs. A dose-related decrease in body weight gain was found. Macroscopical examination revealed effects on the testes only and not on other organs. The absolute and relative weights of the testes and epididymis in the treated rats significantly decreased compared with the control. Histopathology of testes revealed dose-related severe atrophy of the seminiferous tubules with total absence of spermatids and degenerated and desquamated spermatocytes. Examination of the epididymis showed reduced numbers of spermatozoa and increased numbers of degenerated or desquamated cells in the lumen of the epididymal duct. The effects on the epididymis did not show a dose–response relationship, but were more severe with longer dosing, i.e. more severe after 4 weeks than after 2 weeks. In this study, nitrofurazone induced toxicity on the testes and epididymis after oral treatment with 50 and 100 mg/kg b.w. per day for 2 or 4 weeks.

Nishimura et al. (1995) treated male Sprague–Dawley rats (8 weeks old, n = 10) by gavage with nitrofurazone (chemical form not specified, suspended in 0.5 % carboxymethyl cellulose) at doses of 12.5, 25 or 50 mg/kg b.w. per day for 6 weeks, including 4 weeks prior to mating with untreated females during a 2-week period (experiment 1). In addition, groups of 10 male rats (15 weeks old) received doses of 12.5 or 25 mg/kg b.w. per day by gavage for 11 weeks, including 9 weeks prior to mating with untreated females during a 2-week period (experiment 2). Males were sacrificed directly after the mating period and dams were sacrificed on day 14 to 17 of gestation and examined. Mean testes and epididymis weights decreased at 25 and 50 mg/kg b.w. per day in rats treated for 6 weeks. When treated for 11 weeks, mean epididymis weight decreased in both dose groups, and mean testis weight decreased at only 25 mg/kg b.w. per day. Sperm head counts were significantly reduced in the two highest dose groups of experiment 1 and at 25 mg/kg b.w. per day in experiment 2. Histopathological examination of the testes found tubular degeneration and interstitial cell hyperplasia in all animals of the two highest dose groups of experiment 1 and of the highest dose of experiment 2. Failure of spermiation was found in all dose groups: at 28.4 % in the 12.5 mg/kg b.w. per day dose group and at 100 % in the other dose groups in experiment 1, and at 50 % (low dose) and at 100 % (high dose) in experiment 2. None of the dams that were mated with males treated with 50 mg/kg b.w. per day for 6 or 11 weeks or with 25 mg/kg b.w. per day for 11 weeks became pregnant. Only 28.6 % of the dams that were mated with males treated for 6 weeks with 25 mg/kg b.w. per day became pregnant. Pre- and post-implantation losses increased in dams mated with male rats treated for 6 weeks with 25 mg/kg b.w. per day and for 11 weeks with 12.5 mg/kg b.w. per day. In this study, which showed clear effects of nitrofurazone on the reproductive performance of male rats, no NOAEL could be identified.

Male rats (Crj:CD, 8 weeks old) were treated with a single oral dose of 0 (n = 30), 100 (n = 24) or 300 (n = 30) mg nitrofurazone/kg b.w. (Shoda et al., 2001). Three rats for each dose group were sacrificed at different time points post dosing: after 6, 12 or 24 hours, 2 or 4 days and 1, 2 or 4 weeks, and also after 8 or 12 weeks for the highest dose group. In the 300 mg/kg b.w. group, the absolute testis weight significantly decreased from day 4 of treatment to week 8, and the relative weights decreased up to week 12. No effects on testis weights were found at 100 mg/kg b.w. Histopathology

revealed no changes in testes 6 hours after treatment with 300 mg/kg b.w., but, 12 hours after treatment, degeneration of spermatocytes and vacuolisation of Sertoli cells were found. These effects worsened after 24 hours up to 2 weeks post dosing, when atrophy of seminiferous tubules was observed. Regeneration of seminiferous epithelia started 4 and 8 weeks post dosing, although some atrophic and abnormal tubules were still present. In the low-dose group, similar changes in the seminiferous epithelium and vacuolation of Sertoli cells were seen, but the effects were less severe. The same authors measured hormones in the blood (progesterone, testosterone, prolactin, oestradiol, follicle-stimulating hormone (FSH) and luteinising hormone) of male rats (Crj:CD, 9 weeks old) treated orally once with 0 (n = 20) or 300 (n = 22) mg/kg b.w. Animals (five or six in each group) were necropsied 6, 12, 24 or 48 hours after treatment. Sporadic decreased or increased values of the hormones were found, and this was not dependent on time after treatment. Thus, no correlation between the changes seen on degeneration of germ cells and hormone levels could be established in this study.

Male rats (Chester Beatty Research Institute strain, 11 weeks old, six in each group) were fed nitrofurazone (chemical form not specified) for 25 days in the diet at concentrations of 1 500 or 5 000 mg/kg, reduced to 3 000 mg/kg after 3 days (Montemurro, 1960). These concentrations correspond to 180, 600 and 360 mg/kg b.w. per day, respectively, using the default factor of 0.12 as recommended by the EFSA SC (2012). Toxicity in the highest dose group of 600 mg/kg b.w. per day led to a decrease of the dose after 3 days. Weights of the animals at sacrifice decreased dose dependently. Relative testes weights decreased in both treated groups, and relative seminal vesicle weight increased in the low-dose group. Histologically there was no difference in the seminal vesicles between the treated animals and the controls. Testicular atrophy was found in all treated rats, but was more severe in the low-dose group. In the low-dose group, severe degeneration of the spermatogenic epithelium was also found, but no information was reported for the high-dose group.

Nitrofurazone (0.1 % mixed in crushed pellets) was tested for effects on the spermatogenic, endocrine and secretory functions in male Sprague–Dawley rats (60 to 88 days old, n = 3 to 5 for the treated groups and n = 8 for the control group) treated, via the feed, with a dose corresponding to 64 mg/kg b.w. per day for 0, 2, 4, 6, 10, 14 or 28 days (Hagenäs et al., 1978). Pregnant rats were treated with a single dose of cobalt-60 (150 rad) to produce germ cell-depleted male rats, i.e. 'Sertoli cell only' rats (SCO rats). Nitrofurazone caused a time-dependent decrease in the weight of testes in normal rats and a decrease in ventral prostrate in normal and SCO rats treated for 28 days. Significantly increased levels of FSH were found after 10 and 28 days' treatment in normal rats, but not in treated SCO rats, compared with control rats. FSH stimulates androgen-binding protein (ABP) production and it was found that ABP in normal rats decreased until day 6 of treatment and then increased to a maximum after 10 days of treatment; moreover, it was still higher than in the controls after 28 days of treatment. ABP production in SCO rats followed the same pattern as in normal rats, but at a lower level, i.e. at the same level as the SCO control group. As a consequence, nitrofurazone was lethal for all germ cells in normal rats except for some undeveloped primary spermatocytes and spermatogonia after 28 days of treatment. No germ cells exist in the SCO rats, which means that nitrofurazone did not exert its effect on the Sertoli cells. Testosterone levels in serum were not affected in normal and SCO rats. Morphological analyses showed that tubules were severely damaged and that mature germ cells were lacking after 14 and 28 days of treatment. Shorter treatment (2 to 10 days) caused similar effects, increasing with time. Electron microscopy of testes in normal rats revealed that the shape of Sertoli cells was changed and that there was a decreasing number of germ cells. Severe abnormalities were found in surviving spermatids in normal rats treated for 6 and 10 days. Testes from treated SCO rats showed that Sertoli cells were less affected by treatment with nitrofurazone than in normal rats. Furthermore, in both normal and SCO rats, the cell junctions of the inter-Sertoli cells were impermeable for lanthanum, indicating that the blood–testis barrier was not affected by nitrofurazone. All these tests indicate that nitrofurazone mainly affects germ cells.No studies on spermatogenesis were identified for **SEM**, **nifursol** or **DNSH**.

Conclusions

Oral administration of **furazolidone** caused toxic effects on testes and epididymis (200 mg/kg b.w. per day) and on the hypothalamus (50 and 200 mg/kg b.w. per day) in rats treated for 5 days. Cytotoxic effects in testes and reduced relative testes weights were observed in rats treated orally with furazolidone at a dose of 100 mg/kg b.w. per day for 7 days.

No studies on spermatogenesis were identified for **AOZ**.

Cytotoxic effects in testes and reduced relative testes weights were observed in rats treated orally with **furaltadone** at a dose of 100 mg/kg b.w. per day for 7 days.

No studies on spermatogenesis were identified for **AMOZ**.

For **nitrofurantoin**, toxic effects on spermatogenesis were found in rats treated orally for 1 month at doses of 10 or 85 mg/kg b.w. per day.

No studies on spermatogenesis were identified for **AHD**.

Nitrofurazone caused effects on testes and on epididymis in rats at doses of 12.5 to 360 mg/kg b.w. per day orally for up to 12 weeks, and in mice at the only dose tested of 64 mg/kg b.w. per day orally for 10 or 20 days. Cytotoxic effects in testes and reduced relative testes weights were observed in rats treated orally with nitrofurazone at a dose of 100 mg/kg b.w. per day for 7 days.

No studies on spermatogenesis were identified for **SEM**, **nifursol** or **DNSH**.

Based on the available studies, no NOAEL could be identified for toxic effects on spermatogenesis.

8.2.4.2. Embryotoxicity and teratogenicity

Groups of mice (albino C strain) were treated with **furazolidone** (chemical form not specified) in the diet at concentrations of 0 (control, n = 20), 1 000 (days 1 (n = 10), 6 (n = 4) and 10 (n = 3) of pregnancy) or 2 000 mg/kg (day 1 of pregnancy, n = 5) (Jackson and Robson, 1957). Using the default factor of 0.2 as recommended by the EFSA SC (2012), these concentrations correspond to doses of 0, 200 or 400 mg/kg b.w. per day, respectively. Furthermore, mice were treated with doses of 0 (n = 4), 750 (day 6–7, n = 4), 1 000 (days 1 (n = 10), 6–7 (n = 5) and 10–11 (n = 6)), 1 250 (days 1 (n = 10) and 10–11 (n = 3)), 1 500 (day 6–7, n = 3) or 2 000 (day 7, n = 4) mg/kg b.w. per day via gavage in a suspension of olive oil, at various stages of pregnancy (shown in parentheses after the dose). No maternal deaths occurred at doses of 750 or 1 000 mg/kg b.w. per day, but some deaths (25 to 33 %) occurred at the higher doses fed by gavage (1 250, 1 500 and 2 000 mg/kg b.w. per day). Maternal deaths of 60 % were found in the 400 mg/kg b.w. per day dose group treated via feed. The number of abortions and fetal deaths was 90 to 100 % when furazolidone was given on day 1 (200, 400, 1 000 or 1 250 mg/kg b.w. per day), day 6 (200 mg/kg b.w. per day) or days 6 and 7 (1 000 or 1 500 mg/kg b.w. per day) of pregnancy compared with 75 % in the 750 mg/kg b.w. per day dose group fed by gavage on day 6 to 7 of pregnancy and 25 % in the control group. Treatment on day 10 to 11 did not result in increased deaths, except for 33 % in the 1 000 mg/kg b.w. per day dose group treated by gavage. Toxicity in the highest dose group (2 000 mg/kg b.w. per day) treated on day 7 resulted in vaginal bleeding and abortions within 24 hours. At the lower doses, the effects were the same but delayed, with a gradual loss in weight and then resorption of fetuses. Body weight of the litters was lower in all groups treated with furazolidone, but no abnormalities were found.

No embryotoxicity or teratogenicity studies were identified for **AOZ**, **furaltadone** or **AMOZ**.

Mice (ICR/Jcl, 10 to 12 weeks old) were treated s.c. with **nitrofurantoin** (finely ground and suspended in 1 % gelatine solution) in doses of 100 mg/kg b.w. per day (n = 17) or 250 mg/kg b.w. per day (n = 13) on days 9, 10 and 11 of gestation (Nomura et al., 1984). The control group was treated

with a gelatine solution. No effects on fetal deaths and living fetuses were found. Fetus weights significantly decreased in both males and females treated with 250 mg/kg b.w. per day. Malformations, such as cleft palate, syndactyly and oligodactyly were found in 10 out of 131 fetuses treated with 250 mg/kg b.w. per day compared with 1 out of 183 for controls. No malformations were found at 100 mg/kg b.w. per day. No information about maternotoxicity was reported.

Embryotoxicity was studied in rats (Sprague–Dawley) treated with nitrofurantoin (macrocrystals) by gavage in a suspension of 0.5 % aqueous methylcellulose (Prytherch et al., 1984). Male rats were treated with vehicle and divided into three groups (n = 15) or with 10 mg/kg b.w. per day of nitrofurantoin for 60 days before mating. One male was paired with two females until pregnancy was verified. After mating, the males were sacrificed for necropsy and histopathology of the testes. No adverse effects were found and histopathological investigation showed no abnormalities in testes. Females (96 days old, n = 30/group) were treated with 10, 20 or 30 mg/kg b.w. per day for 14 days before mating. Females dosed with 0, 20 or 30 mg/kg b.w. per day were mated with control males from the three vehicle treated groups mentioned above, and females dosed with 10 mg/kg b.w. per day were mated with males dosed with 10 mg/kg b.w. per day. At day 13 of pregnancy, 10 females/group were sacrificed and necropsied. The rest of the females were sacrificed 21 days after weaning for necropsy. No effects on body weights or feed consumption or on clinical signs were found in treated males and females. In dams of the 10 and 30 mg/kg b.w. per day dose groups that had a successful pregnancy, duration of gestation, indicators for fertility, gestation and lactation were normal. In the group treated with 20 mg/kg b.w. per day and sacrificed on day 13 of gestation, an increased number of resorptions was found. For dams in this dose group sacrificed on day 21 of gestation, decreased body weight of live pups at birth and at day 4 and decreased viability of pups at day 4 were found, but no differences in viability of pups between groups were found 21 days after parturition. The only malformations found were hydrocephalus in one male pup in the 10 mg/kg b.w. per day dose group and microphthalmia in one male pup in the 20 mg/kg b.w. per day dose group. Furthermore, rats were bred as described above and females were treated from day 14 of pregnancy until weaning with 10, 20 or 30 mg/kg b.w. per day of nitrofurantoin. Dams were killed at weaning and pups were examined during weaning on days 4, 14 and 21. No treatment-related effects were found in the dams. The only effect found was that treated pups were heavier than those in the control group on certain days. Overall, no dose-related effects were found in these studies in which rats were treated with nitrofurantoin during and before pregnancy. However, as an increased number of resorptions, decreased body weights of live pups at birth and at day 4 postnatally, and decreased viability of pups at day 4 after birth were found in the 20 mg/kg b.w. per day dose group, a NOAEL of 10 mg/kg b.w. per day for embryotoxic effects in rats treated with nitrofurantoin could be identified.

Pregnant Sprague–Dawley rats were treated orally by gavage with nitrofurantoin macrocrystals in a 0.5 % aqueous methyl cellulose suspension at doses of 0 (n = 18), 10 (n = 19), 20 (n = 17) or 30 (n = 20) mg/kg b.w. per day from day 6 to 15 of pregnancy (Prytherch et al., 1984). On day 20 of pregnancy, the dams were killed and mothers and fetuses were examined. No maternotoxicity and no effects on litter size, number of resorptions or fetal weights were found. No major external, visceral or skeletal malformations were found in the fetuses. Some sporadic minor abnormalities were found in a non-dose related manner: 2 out of 105 pups had large anterior fontanelles in the low-dose group, 2 out of 117 pups had missing ribs and short ribs in the high-dose group and 1out of 111 pups had short ribs in the control group. The rate of occurrence of abnormalities in the treated groups (1.90, 0.0 and 1.71 % in the low-, mid- and high-dose groups, respectively) did not differ from that in the control group (1.80 %). A NOAEL for teratogenic effects in rats treated with nitrofurantoin of 30 mg/kg b.w. per day was identified in this study.

Rabbits (New Zealand White) were bred—one male with several females—until pregnancy was verified (Prytherch et al., 1984). Rabbits were treated by gavage with nitrofurantoin macrocrystals in a 0.5 % aqueous methyl cellulose suspension at doses of 0 (n = 17), 10 (n = 15), 20 (n = 12) and 30 (n = 15) mg/kg b.w. per day from day 6 to 18 of pregnancy. On day 29 of pregnancy, the dams were killed and mothers and fetuses were examined. No maternotoxic effects were found. There were no significant differences in litter size, number of resorptions or fetal weight. A non-dose-related

increase in the average number of dead fetuses per dam was found in the treated groups (0.40 (low dose), 0.42 (mid-dose) and 0.20 (high dose)) compared with the controls (0.06). According to the authors, these numbers were not significantly different from the controls. External, visceral and skeletal malformations in the fetuses were not significantly different from those found in the control group. Only sporadic malformations were found: hydrocephalus in 1 out of 129 fetuses in the control group and in 1 out of 141 fetuses in the low-dose group, and fusion of the last thoracic and first lumbar vertebrae and scoliosis in 3 out of 135 fetuses in the high-dose group. A NOAEL for teratogenic effects of 30 mg/kg b.w. per day was identified in this study when rabbits were treated with nitrofurantoin.

No embryotoxicity or teratogenicity studies were identified for **AHD**.

The effects of **nitrofurazone** on teratogenicity were tested in mice (CD-1) (NTP, 1985). Females were mated with one male until pregnancy was verified. The doses were selected based on results of two preliminary toxicity tests where the highest dose, 750 mg/kg feed, caused clear maternotoxicity. Groups (n = 20 to 26) of mice (CD-1, 8–10 weeks old) were therefore treated with 0, 38, 75, 250 or 500 mg/kg in feed, corresponding to reported mean intakes of 0, 6, 14, 41 or 82 mg/kg b.w. per day, respectively, from gestation day 6 to 15. No toxic effects were found in dams on weight gain, body weight, mortality, uterine weight during pregnancy, and absolute and relative liver weight. Sporadically, clinical signs such as lethargy and piloerection were found in dams in all treated groups from day 7 to 16 of gestation. At day 14 to 16 of gestation, sporadic hyperactivity, convulsions and rough coats were found with a tendency, although not significant, for an increase in the highest dose group. No treatment-related effects on corpora lutea or the number of implantation sites per dam were found, but in the two lowest and the highest dose group the percentage of pre-implantation loss decreased. No treatment-related effects on resorptions were found, but a dose-related tendency for an increase in the percentage of dead fetuses per litter and an increase in the percentage of litters with dead fetuses was seen, with higher incidences in the two highest dose groups. No treatment-related effect on the number of non-live implants or adversely affected implants was found, and no effects were found on the number of live fetuses per live litter or on the sex ratio in litters but the fetal body weight per live litter was decreased in the highest dose group only. No increase in malformations in fetuses was found compared to the control group. Nitrofurazone was not teratogenic to mice when given during organogenesis at doses up to 82 mg/kg b.w. per day. For fetotoxicity a NOAEL of 14 mg/kg b.w. per day was identified from this study.

Nitrofurazone (chemical form not specified) was tested in CD-1 mice (n = 50) treated by gavage with a dose of 100 mg/kg b.w. per day from day 6 to 13 of gestation (Hardin et al., 1987). The control group received corn oil. The pups were allowed to live for 3 days. One dam in the treatment group died during the study. The number of viable litters was reduced in the treatment group (28 out of 35) compared with the control group (45 out of 45) and birth weight was reduced in pups from treated dams. The number of live born pups per litter, the percentage survival up to 3 days and weight gain were not affected by treatment. This was a poorly reported study in which 60 chemicals were tested.

Embryotoxicity and malformations in mice (ICR/Jcl, 10–12 weeks old) treated s.c. with nitrofurazone were studied (Nomura et al., 1984). Mice were treated s.c. with nitrofurazone (finely ground and suspended in 1 % gelatine) at doses of 300 mg/kg b.w. once on day 10 of gestation (six mice) or of 100 mg/kg b.w. per day on days 9, 10 and 11 of gestation (16 mice). The control group was treated with a gelatine solution. Increased fetal deaths were found in the group treated on day 10 with 300 mg/kg b.w. The weights of the fetuses significantly decreased for both dose groups. Malformations such as oligodactyly, tail anomalies, polydactyly, defective legs and omphalocoele were found in 1 out of 185 fetuses treated with 100 mg/kg b.w. per day and in 14 out of 66 fetuses treated with 300 mg/kg b.w. of nitrofurazone, compared with in 1 out of 183 fetuses in the control. No information about maternotoxicity was mentioned, but the authors reported that the doses were close to the maximal tolerated dose in mice.

The effects of nitrofurazone on teratogenicity were tested in rabbits (New Zealand White, n = 22 to 27) treated by gavage with nitrofurazone in corn oil at doses of 0, 5, 10, 15 or 20 mg/kg b.w. per day from day 6 to 19 of pregnancy (NTP, 1987). The highest dose was maternotoxic with deaths in 2 out of 26 rabbits, decreased weight gain and increased absolute and relative liver weights. Clinical signs such as lacrimation, glaucoma, weight loss and no faeces occurred sporadically with a tendency to increase with dose and days of pregnancy. However, no clear treatment-related maternotoxicity was found at doses of 5, 10 or 15 mg/kg b.w. per day. No differences in the number of corpora lutea per dam, implantation sites or the percentage of pre-implantation loss per litter were found. No dose-related trend for the percentage resorptions per litter was found for the three lowest dose groups. In the highest dose group, embryotoxicity, characterised by a significant increase in the number of resorptions, the percentage of non-live implants and the percentage of adversely affected implants per litter, was found. The percentage of male fetuses per litter decreased in the highest group. Major anatomical malformations, expressed as a percentage of live fetuses per litter, increased in the highest dose: 14.6 % compared with 2.7 % in the vehicle control. In addition, in the highest dose group, the percentage of litters with malformations (40 % versus 12.5 % for the control group) and the percentage of malformed females per litter (17.1 % versus 1.1 %) increased. No increase in malformations was found in the other treatment groups. Nitrofurazone was not teratogenic to rabbits when given during organogenesis (day 6 to 19 of pregnancy) at doses that were not maternotoxic. From this study, a NOAEL of 15 mg/kg b.w. per day was identified.

Rats (Sprague–Dawley) received **SEM** (aqueous solution) by gavage at doses of 5 (n = 4), 10 (n = 11) or 100 (n = 9) mg/kg b.w. per day on gestation day 12 to 15, or at doses of 25 (n = 3) or 50 (n = 8) mg/kg b.w. per day on gestation day 10 to 16 (Steffek et al., 1972). Maternotoxicity was not reported except that three out of nine rats died from the 100 mg/kg b.w. per day dose group. The only effects studied were resorptions and cleft palate. Increased resorptions were found in groups treated with 50 mg/kg b.w. per day (38 %) and 100 mg/kg b.w. per day (56 %). Cleft palates were found in 12 out of 28, 40 out of 42 and 22 out of 22 fetuses of the 25, 50 and 100 mg/kg b.w. per day dose groups, respectively. No effects on the incidence of cleft palate or resorptions were found at 10 mg/kg b.w. per day. However, because this was the only parameter investigated, the CONTAM Panel could not conclude on a NOAEL for teratogenicity in rats treated with SEM.The effect of SEM on levels of pulmonary surfactants such as phospholipids in new-born rats were investigated (De La Fuente et al., 1983a). Rats (Wistar), using intraperitoneal (i.p.) infusion, were given 100 mg/kg b.w. SEM (aqueous solution) or only distilled water (control group) on day 10 of gestation. Rats (control and treated; n = 8 to 16) were sacrificed on day 18 and day 21 of gestation and one group was allowed to deliver pups, which were studied postnatally on day 1, 3, 7, 15, 22 and 30. Phospholipids were extracted from the lungs of fetuses and offspring. The ratio of phosphatidylcholine to spingomyelin decreased in treated fetuses compared with controls. The ratio was also lower in treated offspring on day 1 after birth, but on day 3 to day 30 the ratio was not significantly different between treated offspring and those in the control group. Based on these results, the authors concluded that SEM has an effect on the surfactants of the lung pre-natally and post natally and could therefore have a negative effect on lung development in offspring from rats treated i.p. with 100 mg/kg b.w. SEM on day 10 of gestation.

De La Fuente et al. (1983b) treated rats (Wistar) i.p. with SEM (dissolved in saline) at doses of 50, 75, 100 or 150 mg/kg b.w. on day 7, 10 or 13 of pregnancy. Control rats received saline only. Rats were either sacrificed on day 21 of gestation or allowed to deliver normally. The pups were studied for 1 month thereafter. Resorptions increased dose dependently, and fetal deaths occurred in all treated groups. The number of live fetuses and fetal weights decreased in all groups except the group treated on day 13 with 50 mg/kg b.w. In all dose groups sacrificed on day 21 of gestation, severe haemorrhages in the liver, brain and intestines, mostly when dams were treated on day 10 (the differentiation period), were found. Furthermore, anophthalmia on one side, cleft palate, absence of testes and hydronephrosis, mostly when animals were treated on day 13 (the development period), were found in the fetuses. Incomplete ossification of the skull, which was most frequent when dams were treated during the implantation period (day 7), was one of the most common abnormalities of the skeleton. Incomplete ossification of sternum and limbs and malformations of the ribs were found in fetuses from all treated groups. Postnatal mortality increased significantly with the dose (25 to 35 % in

the 50 mg/kg b.w. group, 30 to 43 % in the 75 mg/kg b.w. group, 44 to 63 % in the 100 mg/kg b.w. group and 45 to 57 % in the 150 mg/kg b.w. group) compared with control rats (< 5 %) during the first month after delivery.

To test the hypothesis that the teratogenic effect of SEM is caused by the blocking of DNA synthesis, De La Fuente et al. (1983b) measured DNA, RNA and protein in the liver and lungs of Wistar rats treated i.p. with 100 mg/kg b.w. on day 10 of pregnancy. DNA, RNA and protein levels decreased in the lungs of treated rats sacrificed on day 18. When treated rats were sacrificed on day 21, only lung DNA levels decreased compared with control rats. In the liver, protein levels decreased only at sacrifice on day 21 compared with controls. Levels of pulmonary DNA and RNA decreased in fetuses from mothers sacrificed on day 21, but not in offspring. Liver protein levels significantly decreased compared with controls, but liver DNA and RNA levels decreased in only 30-day-old offspring. The authors concluded that the results indicate that the teratogenic effect of SEM might be due to effects on nucleic acids and protein levels in the liver, as alterations in protein levels at the end of pregnancy can alter gestation.

The effect of SEM hydrochloride on rats (Wistar) during intrauterine and postnatal development was investigated (de la Fuente del Rey, 1986). Rats (13–15/group) were treated i.p. with 17 mg/kg b.w. every day during gestation or with doses of 50, 75, 100 or 150 mg/kg b.w. on day 5, 7, 10, 13 or 15 of gestation. Control animals were treated i.p. with saline. Rats were divided into two groups: group A (8 to 10 dams per dose group) rats were killed after 21 days of pregnancy and group B (five dams per dose group) rats were allowed to deliver and the pups were studied for 1 month. High mortality of dams occurred in the highest dose group, mortality was sporadic in the 100 mg/kg b.w. dose group and only one dam died in the 50 mg/kg b.w. group. The numbers of litters, implantations, resorptions and dead fetuses were not affected. The number of live fetuses decreased at all dose levels. Mean fetal weights decreased in all dose groups on all treatment days except when dams were treated on days 13 and 15 in the low-dose group. The only effect found in the group treated with 17 mg/kg b.w. SEM per day was a reduced number of implantations and live fetuses per litter. Abnormalities such as internal hydrocephaly, exencephaly, meningocele, severe haemorrhages in liver and intestines, anophthalmia, cleft palate, absence of testes and hydronephrosis were found in the groups treated with 50, 75, 100 or 150 mg/kg b.w., but they differed in severity in relation to the different days of treatment. Incomplete ossification of the skull, minor malformations in the ribs, absence of sternum and incomplete ossification of limbs were found in all treated groups but not in the control group. Treatment with SEM during the whole gestation period with 17 mg/kg b.w. per day resulted in similar anomalies, except that absence of testes and sternum and incomplete ossification of the limbs did not occur in this group. Postnatal mortality increased in pups from SEM-treated dams on all treatment days and at all doses tested. Maternotoxicity was not reported, but the malformations were found in pups from females that did not show any clear signs of toxicity. Thus, embryotoxic and teratogenic effects were found at all doses, including the lowest dose of 17 mg/kg b.w. per day.

Wiley and Jonega (1978) treated pregnant Golden Syrian hamsters by gavage on day 7 of gestation with 100, 150 or 200 mg/kg b.w. SEM hydrochloride in aqueous solution. All dams of the two highest dose groups died, mostly within 48 hours. Examination of the uterus of the dead dams revealed signs of resorption at all implantation sites. The dams of the 100 mg/kg b.w. group were sacrificed at day 14 of gestation. Mortality, the number of implants and live fetuses, and mean fetal weights were not different from the control group. Growth retardation (weight of less than 60 % of control pups) was found in 16.5 % of the pups, and malpositions of limbs in 5.1 % of the offspring were recorded. No skeletal or visceral abnormalities were found. Maternotoxic effects other than deaths were not reported.

No embryotoxicity or teratogenicity studies were identified for **nifursol** or **DNSH**.

Conclusions

Furazolidone was embryotoxic when given orally to mice at doses of 200 mg/kg b.w. per day or higher during early pregnancy. No NOAEL could be identified for furazolidone in mice.

No embryotoxicity or teratogenicity studies were identified for **AOZ**, **furaltadone** or **AMOZ**.

Nitrofurantoin was not teratogenic or embryotoxic to mice at s.c. doses of 100 mg/kg b.w., but was at 250 mg/kg b.w. per day on days 9, 10 and 11 of gestation. Rats treated orally with nitrofurantoin at doses of 10 mg/kg b.w. per day showed no embryo- or fetotoxic effects, but doses of 20 mg/kg b.w. per day caused effects on resorptions of embryos and led to decreases in body weight and the viability of pups after birth. No teratogenic effects were observed in rats or rabbits treated orally with nitrofurantoin during organogenesis with doses of 10 to 30 mg/kg b.w. per day. A NOAEL for embryotoxicity in rats of 10 mg/kg b.w. per day was identified, and a NOAEL for teratogenicity of 30 mg/kg b.w. per day was identified for both rats and rabbits treated during organogenesis with nitrofurantoin.

No embryotoxicity or teratogenicity studies were identified for **AHD**.

Nitrofurazone tested orally in mice was not teratogenic at doses up to 82 mg/kg b.w. per day. In mice dosed during organogenesis, a NOAEL of 14 mg/kg b.w. for fetotoxicity was identified. Nitrofurazone was not teratogenic in rabbits treated with doses up to 15 mg/kg b.w. per day, but malformations were observed at a dose of 20 mg/kg b.w. per day, which was also maternotoxic. A NOAEL of 15 mg/kg b.w. per day was identified for maternotoxicity in rabbits.

Embryotoxic and teratogenic effects of **SEM** were found at all doses (17 to 150 mg/kg b.w.) tested i.p. in rats during pregnancy. No cleft palate or resorptions were observed in rats treated orally with SEM at a dose of 10 mg/kg b.w. per day. However, as no other effects were studied, a NOAEL cannot be identified.

No embryotoxicity or teratogenicity studies were identified for **nifursol** or **DNSH**.

8.2.4.3. Multigeneration studies

No multigeneration studies were identified for **furazolidone**, **AOZ**, **furaltadone**, **AMOZ**, **nitrofurantoin** or **AHD**.

Nitrofurazone was tested in a two-generation reproduction toxicity study in CD-1 (ICR) BR Swiss albino mice following a continuous breeding protocol (George et al., 1996). A dose-ranging study was performed with mice (11 weeks old, eight mice per sex in each group) treated with nitrofurazone at concentrations of 0, 100, 200, 400, 600 or 900 mg/kg in the diet for 2 weeks. Animals treated with doses $\geq$ 400 mg/kg in the diet showed clinical signs such as hyperactivity, dehydration, inflamed eyelids and excessive circling. No effects on body weight were seen, but water consumption significantly decreased in a dose-dependent manner in the highest dose. Thus, doses lower than 900 mg/kg in feed were used in the following tests.

The F_0 generation of mice (11 weeks old) were treated with nitrofurazone in the feed at concentrations of 0 (n = 40 of each sex), 100 (n = 20 of each sex), 375 (n = 19 of each sex) or 750 mg/kg (n = 18 of each sex), corresponding to 14, 51 or 102 mg/kg b.w. per day, respectively (George et al., 1996). The mice, females and males separated, were first dosed for 1 week. Thereafter, the F_0 mice, living as breeding pairs (one male and one female), were treated with different doses for 14 weeks. After this phase, the F_0 males were separated from the females and their litters, and all mice were treated for an additional 6 weeks, during which time gestation and lactation took place. The litters were euthanised at PND 0. In the highest dose group, fertility was significantly reduced (17 % (3 out of 18 breeding pairs) compared with 95–100 % for the other groups) and the breeding pairs were infertile when the third litter should have been produced. In the mid-dose group (51 mg/kg b.w. per day) fertility in the

fifth litter was 47 % compared with 88 % in controls. The live pup weight was reduced in the high-dose group. In the mid- and high-dose groups, a reduced number of litters per breeding pair, reduced average litter sizes and a decreased percentage of pups born alive were found. No effects were found in the low-dose group. The number of dams showing aberration of labour and/or delivery, and/or inadequate postnatal care, increased dose-dependently by 1 % (control), 3 % (low dose), 14 % (mid-dose) and 25 % (high dose).

Necropsy of the F_0 males at week 27, when animals were 38 weeks of age, showed that liver, kidney and adrenal weights increased in the two highest dose groups compared with controls. Histopathology of male mice revealed hepatic centrilobular hypertrophy at the high dose, and the weights of right corpus, caput epididymis and right testis also decreased in high-dose males. Sperm analysis revealed a decrease in epididymal sperm concentration at the two highest doses and a reduction in testicular spermatid counts in all dose groups. Sperm motility decreased significantly in the highest dose group. Intratesticular testosterone levels increased in the high-dose group only. Histopathology revealed that the incidence of seminiferous tubule degeneration followed by epididymal hypospermia, as well as atrophy, showed treatment-related increases. In females of the high-dose group, decreased body weight and hepatic hypertrophy were found. Altered oestrous cycles were found after examination of vaginal smears in mice of the high-dose group. Relative ovary weights, including oviducts, decreased at all doses, but no histopathology was performed.

Fertility of the F_1 generation was tested in weanling mice reared at PND 21. The mice were fed a diet containing 0, 100 or 375 mg/kg nitrofurazone in the feed, corresponding to 0, 15–21 or 61–80 mg/kg b.w. per day, respectively. In the study, 20 (control), 20 (low dose) and 14 (high dose) mice of each sex were paired until vaginal plugs were found or 1 week had passed. The F_2 generation was delivered and litter data were collected. Reduced fertility and smaller F_2 litters were found in the high-dose group. Clinical signs such as lethargy, hunched back position and dehydration were found in the high-dose F_1 males and females. Reduced testes weight and epididymal sperm number, and abnormal sperm morphology were found in F_1 males. Borderline nephropathy was noted in both dose groups. In F_1 female mice, decreased body weight and liver and ovarian weights were found in the high-dose group, and altered oestrous cycles were found at both doses (George et al., 1996).

The effects on fertility of nitrofurazone as described above were confirmed in a separate cross-over mating study carried out by the same authors (George et al., 1996). To evaluate gender-specific effects, control male and female mice were mated (group 1), high-dose males (102 mg/kg b.w. per day) were mated with control females (group 2) and control males were mated with high-dose females (102 mg/kg b.w. per day) (group 3). Mice (23 weeks old) were mated in breeding pairs until a vaginal plug was detected or 1 week had passed. After delivery (PND 0), all litters were examined and the pups were sacrificed. A significantly reduced mating efficiency (63 % versus 75 % for control) and absence of fertility were found in group 2, and a decreased number of live pups per litter (5.4 versus 10.8 for controls) was found in group 3.

The effect of **SEM** treatment of Wistar rats during gestation, for three successive generations, on hepatic levels of DNA, RNA and protein was investigated by De La Fuente et al. (1983c). SEM in saline was injected i.p. in a single dose of 100 mg/kg b.w. during the 10th day of gestation to the adult females in three successive generations. The different generations were divided into one control group and one treatment group. Livers were collected from sacrificed animals from 21-day-old fetuses, from offspring of 1, 7, 15 or 30 days old and from rats pregnant for 21 days and were analysed for levels of DNA, RNA and protein. A significant decrease of RNA levels was observed for the F_2 and F_3 generations compared with the F_1 generation in 21-day-old fetuses and 1- and 7-day-old offspring. DNA levels were decreased only in the F_3 compared with the F_1 generation in 21-day-old fetuses. No difference in DNA and RNA levels were found for the pregnant rats compared with controls, but if the generations are compared the levels decreased in F_1 and F_2 rats compared with P rats. Hepatic protein levels decreased in the F_2 and F_3 generations compared with the F_1 generation in 21-day-old fetuses. Hepatic protein in pregnant rats decreased in treated rats compared with P and F_1 controls. The authors

suggested that the reduction in nucleic acids and proteins is a possible mechanism for the resorptions and abnormalities found in rats treated with SEM during pregnancy.

A three-generation reproduction study in rats was conducted with **nifursol** (3,5-dinitrosalicylic acid, 5-nitrofurfurylidene hydrazide) in rats (Long–Evans) (Jorgenson, 1967). The parent generation consisted of 60 female and 30 male rats divided evenly into three groups (n = 20 female and 10 males/group) which were treated with nifursol in doses of 0, 400 and 600 mg/kg feed. The doses correspond to 0, 36 and 54 mg/kg b.w. per day, respectively, using the default factor of 0.09 as recommended by the EFSA SC (2012). The parent generation of rats was fed the medicated diet from weaning (3 weeks of age) and was mated at 100 days of age and produced F_{1A} and F_{1B}, of which F_{1B} was fed the medicated diet and mated at 100 days of age and produced the second generation, F_{2A} and F_{2B} generation. F_{2B} was fed the medicated diet and mated at 100 days of age to produce the third generation, F_{3B}, which were necropsied and liver, kidney, gonads and uterus were sampled and analysed for histology. In total, 20 females and 10 males were included in each F group. The F_{1A}, F_{2A} and F_{3A} generations were kept until weaning and then weighed and sacrificed. Fertility was not negatively influenced by treatment with nifursol; instead, a positive effect was found compared with the control group. The ratio of number of dead pups to number of born pups was not influenced by treatment. The litter size and body weights of the treated pups were either higher or equal to the controls. Histology results were not presented, and it was mentioned that no abnormal gross pathology findings were found. Nifursol did not have any effects on reproduction in rats treated with doses up to 54 mg/kg b.w. per day for the three generations.

No multigeneration studies were identified for **DNSH**.

Conclusions

No multigeneration studies were identified for **furazolidone**, **AOZ**, **furaltadone**, **AMOZ**, **nitrofurantoin** or **AHD**.

Reproductive toxicity of **nitrofurazone** administered to mice was clearly shown as a gradual disruption of fertility related to the degeneration of the seminiferous tubules at all doses tested (14, 51 and 102 mg/kg b.w. per day). The most sensitive parameters for males were abnormal sperm morphology and a reduced testicular spermatid concentration. In females, the most sensitive effects were altered oestrous cycles and reduced relative weights of ovaries and oviducts. Nitrofurazone caused reduced fertility in F_0 males and females and in F_1 female mice. The CONTAM Panel concluded that no NOAEL could be identified and that the available study does not provide a reliable basis for establishing a lowest observed adverse effect level (LOAEL) for the reproductive effects of nitrofurazone.

SEM (100 mg/kg b.w. i.p.) decreased hepatic levels of DNA, RNA and protein in the livers of rats treated for three successive generations. The CONTAM Panel concluded that no NOAEL could be identified and that the available study does not provide a reliable basis for establishing a LOAEL for the reproductive effects of SEM.

Nifursol had no effects on reproduction in rats treated with 36 or 54 mg/kg b.w. per day for three generations.

No multigeneration studies were identified for **DNSH**.

8.2.5. Neurotoxicity

No neurotoxicity studies were identified for **furazolidone**, **AOZ**, **furaltadone** or **AMOZ**.

The effect of **nitrofurantoin** on polyneuropathy, a well-known side effect in humans, was investigated in rats (Behar et al., 1965). Sabra rats (20 to 21 per group) were treated with nitrofurantoin (5 % suspension in 0.5 % sodium carboxymethylcellulose) orally in doses of 0, 20, 50

or 100 mg/kg b.w. per day (divided in two doses) for 2, 4, 6 or 8 days. Plasma concentrations of nitrofurantoin were measured daily and were found to be comparable to levels found in humans treated therapeutically. Sciatic and brachial nerves were analysed histopathologically and sciatic nerves were used for examination of functionality by electrophysiological methods, i.e. chronaxie and conduction velocity. Nerves of rats from all dose groups showed axonal dystrophy, independent of dose, which increased with time up to 6 days' treatment. In sciatic nerves, a time- and dose-dependent decrease in conduction velocity was found compared with control groups, and values for chronaxie increased after 2 days' treatment in a time- and dose-dependent manner. This study showed that nitrofurantoin at doses of 20 to 100 mg/kg b.w. per day orally caused both histopathological and electrophysiological abnormalities in peripheral nerves in rats.

Nerve damage was measured in rats (Wistar, Shell strain) treated i.p. with nitrofurantoin (Rose et al., 1982). Two groups of 20–32 rats received doses of 100 or 200 mg/kg b.w. per day for 7 consecutive days. Examination of the animals occurred 2, 3, 4 or 6 weeks after the start of treatment. In the 200 mg/kg b.w. per day group, 18 out of 20 animals were lethargic, showed hunched hindquarters and splayed hind legs before they died. In the low-dose group, there were no deaths, although the animals showed similar clinical signs as in the high-dose group as well as reduced muscle tone. Nitrofurantoin caused increases in β-glucuronidase 2 and 3 weeks after termination of dosing, in sciatic/posterior tibial nerves and in trigeminal ganglia, and in β-galactosidase 3 weeks after the end of dosing in sciatic/posterior tibial nerves compared with control animals. The effect was reversible and returned to control levels after 6 weeks. The same effects were found in the two surviving animals in the high-dose group 3 weeks after the end of treatment. These effects indicate that high doses of nitrofurantoin produce peripheral nerve damage in rats.

No neurotoxicity studies were identified for **AHD** or **nitrofurazone**.

The parenteral dose (CD_{50}) of **SEM** causing convulsions was estimated in different species, e.g. in humans, 40 mg/kg b.w. (i.v.); in monkeys, 60 mg/kg b.w. (i.p.); in rabbits, 175 mg/kg b.w. (i.p.); in rats, 150 mg/kg b.w. (i.p.); and in mice, 111.7 (i.v.) and 116.4 mg/kg b.w. (i.p.). SEM, at a single i.p. dose of 168 mg/kg b.w., was also used as a reference substance for inducing convulsions when testing the effectiveness of anticonvulsants in mice (Jenney and Pfeiffer, 1958).

The effect of SEM hydrochloride on running fits in mice (ddY strain, 4 to 5 weeks old) and its localisation of action were investigated (Yamashita and Hirata, 1977). Mice were injected in the superior colliculus with 2 µL SEM (5 µg/animal or 0.2 mg/kg b.w. for a 25-g mouse), and saline or distilled water were used as controls. The animals were observed for 1 hour before decapitation. Approximately 10 minutes after injection, running fits, in most cases were preceded by hyperactivity and followed by clonic and tonic convulsions. These effects were not observed in the control animals. The anticonvulsant drug pyridoxine, when injected in the superior colliculus or administered i.p., reduced the effects of SEM. In addition, i.p. administration of the anticonvulsant aminooxyacetic acid before SEM treatment reduced the effect of SEM. It was concluded that the effect of SEM on behaviours such as running fits is probably caused by effects in the superior colliculus, which is the centre for visual, auditory and somatosensory inputs.

Yamashita and Hirata (1978) investigated the effect on running fits after intracollicular injection of SEM (2 µL SEM) in the skull of male mice (ddY strain, 4 to 5 weeks old). Mice were treated with 3, 5, 6, 7, 8, 10 or 20 µg/animal (n = 3 to 5, except for the middle dose, n = 10). Saline or distilled water were used as controls. The mice were observed for 1 hour before being sacrificed. A dose of 6.4 µg/animal induced running fits in 50 % of the animals. Running fits started at about 10 minutes after injection and were repeated often. Tonic and clonic convulsions often occurred after the running fits, and they sometimes caused death of the mice. The mice injected with SEM intracollicularly showed hypersensitivity to sounds. Pyridoxine and aminooxyacetic acid inhibited the effect on running fits of SEM, as was also shown in the previous study. This study confirmed the results of the previous study by Yamashita and Hirata (1977).

Male mice (ddY strain, 4–5 weeks old) were injected with SEM dissolved in 5 µL of water or saline in the left lateral ventricle (intracerebrally (i.c.)) (Yamashita, 1976). SEM was injected at doses of 0, 30 (n = 10), 35 (n = 5), 40 (n = 5), 50 (n = 10) or 60 (n = 20) µg/mouse. In the 50 and 60 µg/mice groups, 5 out of 10 and 11 out of 20 of the rats died, respectively. The latent period for the first convulsions decreased with dose: it was 22 minutes in the lowest dose and 13 minutes in the highest dose group. SEM caused tremors and tonic and clonic convulsions in treated mice. It was also shown that mice fed a vitamin B_6-deficient diet initiated tremors and convulsions at lower doses of SEM than mice fed a normal diet. Vitamin B_6 injected i.c. at the same time that 30 µg/mouse of SEM was injected i.c. prevented convulsions in the mice. When higher doses of SEM were used and injected i.c. but pyridoxine (a form of vitamin B_6) was injected i.p., the number of convulsions increased. SEM was also injected in different sites of the brain at a dose of 20 µg/mice. It was concluded that SEM might act on two sites of the brain, one related to running fits (adjacent to the lateral ventricle) and one related to convulsions and tremors (midbrain).

The effect of SEM on development and maturity was tested in male juvenile rats (n = 6 to 8) treated orally with 40 or 75 mg/kg b.w. per day at PND 51 to 60 (Maranghi et al., 2009). An open field test was performed. Rats treated with SEM were less active, had less locomotor activity and had a markedly decreased frequency of crossing than the control rats. Wall rearing and grooming increased in the SEM-treated rats. In addition, a plus maze test was done showing a low level of locomotor activity and a decreased frequency of stretched attended postures in SEM-treated rats. No NOAEL for these effects was identified.

No neurotoxicity studies were identified for **nifursol** or **DNSH**.

Conclusions

No neurotoxicity studies were identified for **furazolidone**, **AOZ**, **furaltadone** or **AMOZ**.

Nitrofurantoin caused peripheral nerve damage in rats treated orally (20 to 100 mg/kg b.w. per day) and i.p. (100 or 200 mg/kg b.w. per day). The CONTAM Panel concluded that these studies cannot serve as a basis for identifying a NOAEL for neurotoxic effects of nitrofurantoin.

No neurotoxicity studies were identified for **AHD** or **nitrofurazone**.

SEM caused less locomotor activity, decreased curiosity and increased grooming when juvenile rats were treated orally with 40 or 75 mg/kg b.w. per day for 10 days. SEM injections in the brains of mice caused convulsions and behavioural changes, such as increased running fits, at a dose of 0.2 mg/kg b.w. The CONTAM Panel concluded that these studies cannot serve as a basis for identifying a NOAEL for neurotoxic effects of SEM.

No neurotoxicity studies were identified for **nifursol** or **DNSH**.

8.2.6. Genotoxicity

Genotoxicity of **furazolidone** has been extensively tested in a variety of studies. Positive findings were recorded in bacterial reverse mutation assays with *S.* Typhimurium TA100, TA98, TA98NR and TA98/1,8-DNP_6 and *E. coli* strains WP2, WP2s and TC3960, with and without metabolic activation. In *E. coli* PQ97 and *S.* Typhimurium TA1535/pSK1002, furazolidone induced SOS response. It was positive in the sex-linked recessive lethal test in *Drosophila melanogaster*, and it induced gene mutations in mammalian cells *in vitro*. Positive results were obtained in most of the *in vitro* chromosome aberration assays, sister chromatid exchange (SCE) assays and micronucleus assays. In a human lymphoblastoid cell line (TK6) (Borroto et al., 2005) and in human hepatoma HepG2 cells (Jin et al., 2011), furazolidone induced DNA strand breaks. In the latter study, it was shown that furazolidone also induced an increase in ROS and formation of 8-hydroxydeoxyguanosine adducts indicating involvement of oxidative stress (Jin et al., 2011). In the *in vivo* bone marrow SCE assay in mice, furazolidone gave a positive result at doses ≥ 30 mg/kg b.w. (Madrigal-Bujaidar et al., 1997). Of

the two *in vivo* mouse micronucleus tests with furazolidone, one was negative while another gave equivocal results. Details and references for these studies are shown in Appendix H, Table H.1.

The metabolite of furazolidone, **AOZ**, was tested in the reverse mutation assay with *S.* Typhimurium strains TA98, TA100, TA1535 and TA1537, and *E. coli* WP2uvrA. It was positive in TA1535, TA100 and WP2 in the presence of metabolic activation, while in the absence of metabolic activation it was positive in TA1535 only (NOTOX, 1994f; Hoogenboom et al., 2002) (Appendix H, Table H.2). In stimulated human lymphocytes, it induced a dose-dependent increase in chromosomal aberrations in the absence of metabolic activation (NOTOX, 1994d; Hoogenboom et al., 2002). The *in vivo* micronucleus assay with AOZ was performed on Swiss mice using single i.p. administration and sampling after 24 and 48 hours using five animals per dose and sampling time (NOTOX, 1994h). Owing to the higher susceptibility of males than females to the toxicity of AOZ, males were administered 32–500 mg/kg b.w. AOZ and females were administered 270–1 500 mg/kg b.w. A statistically significant increase of micronucleated polychromosome erythrocytes (PCEs) was detected at only 48 hours of sampling in the males of the highest dose group. Large differences in toxicity and mortality, as well as in the frequency of micronucleated PCEs, were observed, indicating a possible non-genotoxic mechanism of action.

Only a few genotoxicity studies of **furaltadone** and its metabolite AMOZ were available to the CONTAM Panel. In a bacterial reverse mutation assay with *S.* Typhimurium TA100 without metabolic activation, furaltadone was already positive at the lowest tested concentration of 10 ng/plate, whereas the N-oxide metabolite was negative at doses up to 1 000 ng/plate (Hoogenboom et al., 1994). In the mouse lymphoma assay, furaltadone (concentration range: 10–1 000 µg/mL) induced a 4- to 18-fold increase in the mutant frequency at the thymidine kinase (TK) locus in the absence of metabolic activation, and a five- to six-fold increase in the presence of metabolic activation (RRC NOTOX, 1991a). However, there are no data on its clastogenic activity. In the bacterial reverse mutation assay with *S.* Typhimurium TA1538, TA98, TA1535 and TA100 and *E. coli* WP2uvrA, **AMOZ** was negative in the presence and in the absence of metabolic activation (NOTOX, 1994e). AMOZ was not clastogenic in the *in vitro* chromosomal aberration assay with and without metabolic activation (NOTOX, 1994c).

In bacterial genotoxicity and mutagenicity tests, **nitrofurantoin** induced DNA single strand breaks in nitroreductase-rich, but not nitroreductase-deficient, *E. coli* strains. Induction of reverse mutations was detected in *E. coli* strains WP2 and WP2uvrA and *S.* Typhimurium TA100, TA98 and TA97, but not in TA1535, TA1536 or TA1538. In yeast (*Saccharomyces cerevisiae*), it induced mitotic gene conversions in D4-RDII and D7 strains, but not in the D4 strain and non-disjunction. In a diploid *Aspergillus nidulans*, nitrofurantoin induced mitotic crossing-over. In Chinese hamster cells, nitrofurantoin induced resistance to 6-thioguanine only in the presence of metabolic activation. Nitrofurantoin induced DNA strand breaks in human foreskin fibroblasts and in a human lymphoblastoid cell line (TK6) *in vitro* and increased the frequency of SCE and chromosomal aberrations in isolated human lymphocytes *in vitro*. *In vitro*, nitrofurantoin did not induce unscheduled DNA synthesis (UDS) in human fibroblasts or rat hepatocytes. Details and references for these studies are shown in Appendix H, Table H.3.

In *D. melanogaster*, the result of the sex-linked recessive lethal test was negative, but positive results were observed in the wing spot test (Appendix H, Table H.3).

In vivo, nitrofurantoin was negative in the mouse spot test and in the dominant lethal test in mice (Appendix H, Table H.3). It also did not induce chromosomal aberrations in male germ cells or dominant lethal effects in mice, whereas it induced DNA strand breaks in different organs in rats and mice and SCEs in bone marrow cells of mice (Appendix H, Table H.3).

In an *in vivo* micronucleus study, nitrofurantoin was administered (5, 10 or 50 mg/kg b.w.) to young (3-week-old) and adult (8-week-old) BALB/C mice with a single i.p. injection. The blood samples for the micronucleus analysis were collected 48, 96, 168 and 336 hours after the administration. A

significant increase in micronuclei frequency was observed at all doses of nitrofurantoin, with higher levels in young animals than in adult animals. The peak level was observed after 48 hours and then gradually declined. In adult animals, the micronuclei frequency declined to the background level, whereas in young animals it remained elevated (Fucić et al., 2005). The study indicates that young animals are more sensitive to the genotoxic effects of nitrofurantoin than adult mice and that the response in young mice persists for a significantly longer time.

In vivo mutagenicity testing of nitrofurantoin was performed with Big Blue transgenic mice (Quillardet et al., 2006). The male Big Blue^{TM} C57BL/6[LIZ] mice received 167 mg/kg b.w. per day by gavage for 5 consecutive days. The animals were sacrificed 20 days after the last administration. The frequencies of mutants in the cII gene from the shuttle vector were determined in lung, kidney, bladder, caecum, colon, intestine, kidney, spleen and stomach. A weak mutagenic response was observed in all organs; the highest was in kidney of nitrofurantoin-treated mice.

Kijima et al. (2015) investigated *in vivo* mutgenicity of nitrofurantoin, its constituent compound 5-nitro-2-furaldehyde and its metabolite AHD in F344 gpt delta male rats. The animals were exposed to the tested compounds by gavage at a carcinogenic or the maximum tolerated dose and sacrificed at 4 or 13 weeks. An increase in gpt mutant frequency was observed in nitrofurantoin and 5-nitro-2-furaldehyde treated groups, but not in the AHD-treated group. A significant increase of the 8-hydroxydeoxyguanosine level in kidney DNA was observed after 4 weeks in nitrofurantoin treated rats, but not in 5-nitro-2-furaldehyde or AHD treated rats. In the nitrofurantoin group and to a lesser extent also in the 5-nitro-2-furaldehyde treated group, the accumulation of hyaline droplets in the proximal tubules that stained positive for α2u-globulin were observed. It is known that the accumulation of α2u-globulin causes the proximal tubular cell injury that leads subsequently to compensatory cell proliferation (Borghoff et al., 2001), which may be a mechanism of non-genotoxic carcinogenicity of α2u-globulin inducing compounds. A sencond expriment with nitrofurantoin was performed using female gpt delta rats, in which the effects of α2u-globulin were not involved. The administration of nitrofurantoin at the same dose used for males caused significant elevations of both gpt mutation frequency and 8-hydroxydeoxyguanosine levels to the same extent as in males indicating that α2u-globulin -mediated nephropathy due to nitrofurantoin treatment did not affect susceptibility to nitrofurantoin-induced genotoxicity.

Nitrofurantoin-induced genotoxic effects have also been studied in human patients treated with nitrofurantoin. Sardas et al. (1990) found no increase in SCE frequency in the blood of 15 adult patients with urinary tract infection that were treated daily with oral doses of 10 or 400 mg nitrofurantoin for 10 days. Slapsyte et al. (2002) determined the frequencies of chromosomal aberrations and SCE in children that were under long-term prophylactic treatment with nitrofurantoin. A total of 69 0.2- to 13-year-old children treated with nitrofurantoin at a dose of 5–8 mg/kg b.w. per day for the first 7 days and 1–2 mg/kg b.w. per day for the rest of the treatment period were included in the study. Blood sampling was performed before the therapy and after 1, 3, 6 and 12 months. However, for only 13 patients were blood samples available before the treatment and after 1–12 months. All patients had also undergone X-ray examination (urethrocystography) prior to the treatment. The only effect was a higher frequency of chromosomal aberrations due to X-ray examination. A significant increase in SCE frequency was observed in the group of children from whom blood samples were available both before and after the treatment only, and a significant correlation was observed between cumulative dose of nitrofurantoin and SCE frequency in the lymphocytes of children treated for 1 month. A similar effect was also observed in the group of children after 12 months of the treatment; however, the correlation was not significant, probably owing to the small sample size.

The results of the the *in vivo* mutgenicity study in F344 gpt delta male rats showed that **AHD** was not mutagenic (Kijima et al., 2015). No other genotoxicity studies were identified for AHD.

Nitrofurazone induced differential toxicity in *E. coli*, but not in *S.* Typhimurium. It induced mutations in *E. coli* WP2 and WP2uvrA strains and in *S.* Typhimurium TA98, TA100 and TA1535 strains in the

presence and absence of metabolic activation, while it was negative in *S.* Typhimurium strains TA1536, TA1537 and TA1538. Nitrofurazone induced mutations in *Neurospora crassa*, but not in *A. nidulans*, while in *D. melanogaster* it did not induce sex-linked recessive lethal mutations. In Chinese hamster ovary (CHO) cells, it induced HGPRT mutations and chromosomal aberrations in the presence and absence of metabolic activation. In human, hamster and mouse cells, nitrofurazone induced DNA strand breaks. It did not induce UDS in human cells. Contradictory results were obtained on the induction of chromosomal aberrations in mammalian cells. *In vivo*, nitrofurazone did not induce chromosomal aberrations in rats, micronuclei in mice or rats or sperm abnormalities in mice. Details and references for these studies are shown in Appendix H, Table H.4.

Various genotoxicity studies focused on the nitrofurazone marker metabolite **SEM** (see also Appendix H, Table H.5). In the *S.* Typhimurium reversion test with and without metabolic activation (S9 from Aroclor-induced rat liver and from Aroclor-induced mouse liver and lung), SEM showed weak mutagenic activity in strain TA1535 only. The mutagenic response was partially and totally depressed in the presence of liver and lung S9, respectively. With strains TA1537, TA1538, TA98 and TA100, negative results were obtained, either with or without S9 (De Flora, 1981; De Flora et al., 1984). Negative results were obtained in a study using a modified test protocol with *S.* Typhimurium strains G46, C3076, D3052, TA1535, TA1537, TA1538, TA98 and TA100 and the *E. coli* strains WP2 and WP2uvrA, with and without metabolic activation by Aroclor-induced rat liver S9 (no experimental details given) (McMahon et al., 1979). SEM hydrochloride tested with *S.* Typhimurium strains TA1535, TA1537, TA98, TA100 and TA102 at doses ranging from 50 to 5 000 µg/plate with and without exogenous metabolic activation showed a weak mutagenic response only for the strain TA1535 at the highest tested concentration and only in the absence of S9 (Herbold, 2003).

SEM has been tested more recently in a series of genotoxicity tests including bacterial reverse mutation assays with *S.* Typhimurium strains TA1535, TA1537, TA98 and TA100 and *E. coli* WP2uvrA for *tk* locus mutations with mouse lymphoma cells (L5178Y) and for chromosomal aberrations with CHO cells (TNO, 2004a, b, c). In the bacterial mutagenicity testing (concentration range: 62–5 000 µg/plate), strain TA1535 showed a dose-dependent increase of revertant colonies. In the absence of metabolic activation, a 16-fold increase in the number of revertants compared with the control was observed, while in the presence of metabolic activation the increase was only two-fold. Borderline mutagenicity was also observed in strain TA100, but only without metabolic activation (TNO, 2004a). No mutagenicity was observed in the other bacterial strains (EFSA, 2005). In the *in vitro* forward mutation assay at the *tk* locus with L5178Y cells, SEM (tested at concentrations ranging from 0.21 to 10.0 mM) was positive in the absence of metabolic activation, while in the presence of metabolic activation only a borderline increase in mutant colonies was observed at the highest dose (TNO, 2004b). In the chromosomal aberration assay with CHO cells exposed to SEM for different periods of time (4, 18 and 32 hours), and with sampling after 18 or 32 hours, the compound did not significantly increase the number of aberrant cells, neither with nor without metabolic activation (TNO, 2004c). However, with metabolic activation, an increase in endoreduplicated cells was observed at the early sampling time (18 hours), indicating alterations in the cell cycle control rather than genotoxicity. In a study with Chinese hamster V79 cells, SEM hydrochloride was tested at concentrations ranging from 125 to 1 120 µg/mL. It was cytotoxic at concentrations above 800 µg/mL after 4 hours' exposure and at concentrations above 125 µg/mL after 18 hours' exposure. With metabolic activation, no cytotoxicity was observed. Chromosomal aberrations were determined at concentrations of 250–1 120 µg/mL with and without metabolic activation after 4 hours' exposure and at concentrations of 125–500 µg/mL in the presence of metabolic activation. No significant increase in the number of aberrant metaphases was observed (Herbold, 2004). Recently, Vlastos et al. (2010) investigated the genotoxicity of SEM *in vitro* with human lymphocytes. SEM was tested at concentrations of 0.5–20 µg/mL and revealed a slight increase in SCE frequency only at the highest concentration. No increase in micronuclei frequency was found *in vitro* with human lymphocytes tested at concentrations of 0.5–20 µg/mL.

The *in vivo* micronucleus study with two strains of mice (male BALB/C and CBA) that received a single i.p. dose of SEM (40, 80 or 120 mg/kg b.w.), followed by blood analyses with the sensitive flow

cytometry determination of micronuclei frequency 42 hours after administration, revealed negative results in both strains (Abramsson-Zetterberg and Svensson, 2005). In an *in vivo* study, SEM did not induce DNA damage (UDS) in liver of female mice that received a single oral dose (100 or 200 mg/kg) with sampling times 4 and 16 hours after the administration (CTL, 2004). However, in a recent *in vivo* study with male Wistar rats exposed to SEM (single oral dose of 50, 100 or 150 mg/kg b.w.) and sacrificed after 24 hours, the analysis of bone marrow polychromatic erythrocytes revealed a significant increase of slightly more than two-fold in micronuclei frequency at all doses, but with no dose–response pattern (Vlastos et al., 2010). It should be noted that these doses are relatively high and similar. The discrepancy between the *in vivo* genotoxicity studies in mice (Abramsson-Zetterberg and Svensson, 2005; CTL, 2004) and the rat study (Vlastos et al., 2010) might be the result of the differences in responses between species and remains to be clarified.

No data on the genotoxicity of **nifursol** were identified in the open literature. Therefore, EFSA requested access to the original study reports submitted to SCAN for the risk assessment of nifursol in 2001 and 2003 (see Documentation provided to EFSA). In the bacterial reverse mutations assay with *S.* Typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100, nifursol was clearly positive in the TA100 strain with and without metabolic activation and in the TA98 strain without metabolic activation. In Chinese ovary cells, nifursol induced a consistent but insignificant increase in chromosomal aberrations without metabolic activation at the maximal soluble concentrations, whereas in the isolated rat hepatocytes it did not induce UDS. The results of *in vivo* chromosomal aberration and micronucleus assays in bone marrow were negative. An *in vivo* UDS assay in liver was negative, whereas in intestinal tissue it was positive at higher concentrations. Irritation of the tissue was observed at these concentrations and therefore it is not clear whether the increased incorporation of tritiated thymidine is the consequence of UDS or irritation-induced scheduled DNA synthesis. In the *in vivo* transgenic mutation assay with Muta-Mouse, no increase in the frequency of the lacZ vector was detected in the ileum/jejunum. Other tissues were not analysed. Details and references for these studies are shown in Appendix H, Table H.6.

No genotoxicity studies were identified for **DNSH**.

Conclusions

Table 8 summarises the results from the available genotoxicity studies for the nitrofurans and their marker metabolites considered in this opinion.

Furazolidone has been shown to induce mutations in bacteria and insects. In mammalian cells *in vitro*, it caused chromosomal aberrations, SCE and DNA strand breaks. *In vivo* micronucleus studies gave negative or equivocal results, while *in vivo* it induced an increase in SCE frequency. The CONTAM Panel concluded that these data provide sufficient evidence to show that furazolidone is genotoxic *in vitro*. Based on the limited *in vivo* data, furazolidone may possibly be genotoxic *in vivo*.

The limited data on the genotoxicity of **AOZ** indicate that it is genotoxic *in vitro* and possibly also *in vivo*.

The genotoxicity studies of **furaltadone** indicate that it is a strong bacterial mutagen and induces mutations in mammalian cells, but no study is available on its clastogenicity. The CONTAM Panel concluded that it is mutagenic *in vitro*.

The genotoxicity data on **AMOZ** indicate that it is not genotoxic *in vitro*.

Nitrofurantoin induced DNA damage and mutations in different bacterial test systems, as well as in insects. In mammalian cells *in vitro*, it induced mutations, DNA damage and chromosomal aberrations. *In vivo*, nitrofurantoin has been shown to induce DNA damage in multiple organs, micronuclei formation in mice and gene mutations in a transgenic mouse mutation assay. The study in children under long-term prophylactic treatment with nitrofurantoin gave an indication of the possible

induction of SCE in lymphocytes. Based on these data, the CONTAM Panel concludes that nitrofurantoin is genotoxic *in vitro* and *in vivo*.

The only available mutagenicity study for **AHD** was a negative study with F344 gpt delta rats. Based on these limited data, it is not possible to draw a conclusion about the genotoxicity of AHD.

Nitrofurazone was genotoxic and mutagenic in bacteria and fungi. In mammalian cells *in vitro*, it induced DNA damage, SCE, chromosomal aberrations and mutations, although negative results were also obtained in some studies. In the *in vivo* genotoxicity tests in rodents, nitrofurazone was not genotoxic. Based on these data, the CONTAM Panel concludes that there is sufficient evidence to consider nitrofurazone as genotoxic *in vitro*. Owing to the lack of *in vivo* mutagenicity tests, no conclusion on the *in vivo* genotoxicity can be drawn.

The data on *in vitro* genotoxicity indicate that **SEM** is mutagenic in bacteria and mammalian cells. Earlier studies showed that SEM is not clastogenic *in vitro* or *in vivo.* Based on these data, the AFC Panel (EFSA, 2005) concluded that the weak genotoxicity exerted by SEM *in vitro* is not expressed *in vivo*. The study by Vlastos et al. (2010), which was performed after the EFSA evaluation of SEM, showed the *in vivo* clastogenic potential of SEM. However, owing to the lack of a dose–response relationship, the result cannot be considered as clearly positive and remains to be clarified. Based on the available information, the CONTAM Panel concludes that there is sufficient evidence to conclude that SEM is genotoxic *in vitro*, but that no conclusion on genotoxicity *in vivo* can be drawn.

The data on *in vitro* genotoxicity indicate that **nifursol** is mutagenic in bacteria, and the chromosomal aberration tests in mammalian cells are equivocal. The *in vivo* clastogenicity studies gave clear negative results, as did the *in vivo* mutation assay with transgenic mice. Based on these data, the CONTAM Panel concludes that nifursol is mutagenic *in vitro* but not genotoxic *in vivo*.

No genotoxicity studies were identified for **DNSH**.

Table 8: Summary of genotoxicity testing results in bacteria and in *in vitro* and *in vivo* mammalian test systems

Compound	Bacteria	Mammalian cells *in vitro*						Rodents *in vivo*						Evaluation[c]
	Mut	DSB	SCE	UDS	CA	MN	Mut	DSB	UDS	SCE	CA	MN	Mut[b]	
Furazolidone	P	P	P	N	P	P	P	–[a]	–	P	–	N/E	–	Genotoxic *in vitro* and possibly also *in vivo*
AOZ	P	–	–	–	–	P	–	–	–	–	–	P[d]	–	Genotoxic *in vitro* and possibly also *in vivo*
Furaltadone	P	–	–	–	–	–	P	–	–	–	–	–	–	Mutagenic *in vitro*
AMOZ	N	–	–	–	N	–	–	–	–	–	–	–	–	Non genotoxic *in vitro*
Nitrofurantoin	P	P	P	N	P/N	–	P	P	–	P	–	P/N	P	Genotoxic *in vivo*
AHD	–	–	–	–	–	–	–	–	–	–	–	–	N	Not mutagenic *in vivo* but no other genotoxicity data available
Nitrofurazone	P	P	P	–	P/N	–	P	–	–	N	N	N	–	Genotoxic *in vitro*
SEM	P	–	(P)	–	N	N	P	–	N	–	–	(P)/N	–	Genotoxic *in vitro*
Nifursol	P	–	–	N	E	–	–	–	N/P	–	N	N	N	Mutagenic *in vitro* but not genotoxic *in vivo*
DNSH	–	–	–	–	–	–	–	–	–	–	–	–	–	–

CA: chromosomal aberrations; DSB: DNA strand breaks; E: equivocal; MN: micronucleus; Mut: mutations; N: negative result; P: positive result; (P): weak effect; SCE: sister chromatid exchange; UDS: unscheduled DNA synthesis.
(a): Not tested.
(b): Studies with transgenic rodents.
(c): The results were evaluated in accordance with the EFSA opinion on the strategies for genotoxicity testing (EFSA SC, 2011).
(d): Possible non-genotoxic mechanism of action.

8.2.7. Chronic toxicity and carcinogenicity

The CONTAM Panel identified four carcinogenicity studies on **furazolidone** that had been submitted by industry to JECFA for the evaluation of furazolidone (FAO/WHO, 1993a). With the exception of the study with Fischer 344 rats (see below), the original documents were made available by the data owner. For the study with Fischer 344 rats, the CONTAM Panel used the JECFA summary (FAO/WHO, 1993a). In one study that had been submitted by industry to JECFA for the evaluation of nitrofurazone (FAO/WHO, 1993b), furazolidone was also applied. This study (Siedler and Sierfoss, 1966; see below) was made available to EFSA by the data owner. No carcinogenicity studies on furazolidone were identified in the public literature.

Groups of Swiss MBR/ICR mice (50/sex/group) were fed diets containing 0, 75, 150 or 300 mg/kg furazolidone (purity not given), equal to a reported average daily dose of about 0, 12, 24 or 47 mg/kg b.w. per day, respectively, for 13 months. After the treatment period, the animals were kept on control diets for an additional 10 months. No substance-related effects were observed on feed consumption and body weight. Mortality had increased in mid- and high-dose females at the end of the treatment period of 13 months (8 out of 50 (hereafter in this section '8/50'), 4/50, 12/49 and 12/49 for control, low-, mid- and high-dose groups, respectively). At the end of the study (month 23), mortality had increased in mid- and high-dose females (33/50, 35/50, 46/49 and 48/49 for control, low-, mid- and high-dose groups, respectively) and in high-dose males (34/50, 34/50, 36/50 and 48/51 for control, low-, mid- and high-dose groups, respectively). The incidence of bronchial adenocarcinomas significantly increased in both sexes (incidences in the control, low-, mid- and high-doses groups were 13/49, 19/48, 26/50 and 37/51 for males, and 15/50, 18/50, 20/47 and 30/48 for females, respectively). The incidence of lymphosarcomas significantly increased in mid- and high-dose males (1/49, 7/48, 10/50 and 10/50 for the control, low-, mid- and high-dose groups, respectively) (Halliday et al., 1974).

Groups of 35 female Holtzman rats were fed diets containing 0 or 0.1 % furazolidone (purity not given), corresponding to reported average doses of 0 or 57 mg/kg b.w. per day, respectively, for 45 weeks, followed by a control diet for 8 weeks. At termination (week 53), survival was significantly reduced by the treatment: 19/35 mice survived in the treatment group, whereas 33/35 survived in the control group. In the treatment group, the incidence of animals with mammary adenomas was 21/35, whereas it was 2/35 in the controls, and the incidence of adenocarcinomas was 5/35 in the treatment group compared with none in the controls (Siedler and Searfoss, 1966).

Groups of Fischer 344 rats (50/sex/group) were fed diets containing 0, 250, 500 or 1 000 mg/kg furazolidone (purity not given) for 20 months (corresponding to 0, 12.5, 25 or 50 mg/kg b.w. per day, respectively). The surviving rats were maintained on control diets for at least 4 months or until 90 % of the rats had died. Extensive histopathological examinations were performed on all moribund and sacrificed rats. In the mid-dose (males) and high-dose (males and females) groups, the mortality rate increased after 24 months, with 90 % mortality in the highest dose group. At the end of the treatment period, body weight gain had significantly decreased at mid- and high-dose animals (no numerical data reported by JECFA) (FAO/WHO, 1993a). In the high-dose group, the number of erythrocytes significantly decreased in males and females. Haemoglobin levels and haematocrit decreased in mid- and high-dose females. The incidence of testicular atrophy increased in mid- and high-dose males and the incidence of adrenal cortical hyperplasia increased in high-dose males only. Particularly in the mid- and high-dose groups, increased incidences of dermal fibromas, sebaceous adenomas and thyroid adenomas were observed in both sexes, and increased incidences of basal cell epitheliomas in males were also observed. In 2/50 males of the high-dose group, basal cell carcinomas were found. In female rats, an increased incidence of mammary neoplasms (benign and malignant combined) was observed at all dose levels, but without a dose–response relationship (11/49, 29/50, 40/50 and 30/50 for control, low-, mid- and high-dose groups, respectively). Mammary adenocarcinomas were found in only high-dose females (6/50) (King et al., 1972b; Halliday et al., 1973b).

Groups of Sprague–Dawley rats (35/sex/group) were fed diets containing furazolidone for 2 years. The actual average dose over the 2 years was reported as 0, 0.7, 3.4 or 10.4 mg/kg b.w. per day for males

and 0, 0.8, 4.3 or 14 mg/kg b.w. per day for females. At the end of the study, survival had decreased in female rats, particularly in the high-dose group (26/35, 22/35, 23/35 and 15/35 for control, low-, mid- and high-dose groups, respectively). At termination of the study (day 726), a clear dose-related reduction in red blood cell parameters (red blood cell count, haematocrit and haemoglobin) was observed in mid- and high-dose female rats. A similar but smaller effect was seen at earlier sampling times. In males and females of the high-dose group, a significantly increased incidence of adrenal cortical hyperplasia was observed. In females, an increase in thyroid atrophy was observed in 1/33 animals of the mid-dose group and 8/35 animals of the high-dose group, but not in the control and low-dose group. In females, an increased incidence of malignant mammary tumours was observed (unspecified malignant mammary tumours: 1/34, 3/35, 4/33 and 4/35; adenocarcinomas: 1/34, 2/35, 2/35 and 3/35; carcinosarcomas: 0/34, 1/35, 2/33 and 1/35; for the control, low-, mid- and high-dose groups, respectively). In addition, the incidence of mammary fibroadenomas increased in treated animals, but without a clear dose–response relationship. The authors reported that the time of onset of mammary neoplasms was approximately 2 months earlier in the mid- and high-dose females than in the other groups (King et al., 1972a; Halliday et al., 1973a).

Sprague–Dawley rats (50/sex) were fed diets containing 0, 250, 500 or 1 000 mg/kg furazolidone (purity not given) for 18 months (corresponding to 0, 12.5, 25 or 50 mg/kg b.w. per day, respectively, when applying a default factor of 0.05 (EFSA SC, 2012)). After the treatment period, the animals were maintained on a control diet. A dose-related increase in mortality was found in both sexes: 4/50, 11/50, 17/50 and 30/50 for males and 9/50, 12/50, 8/50 and 29/50 for females, for control, low-, mid- and high-dose groups, respectively. High-dose animals were sacrificed at day 666, whereas controls were sacrificed at day 895. Body weight gain significantly decreased in mid- and high-dose males and in high-dose females. These effects were accompanied by reduced feed consumption in these dose groups. A reduction in the number of red blood cells was seen in female rats, particularly in the mid- and high-dose groups. Histopathological examination showed an increased incidence of hepatic necrosis in all treated rats, but particularly in high-dose females: incidences were 1/50, 3/49, 2/50 and 5/49 for males and 1/49, 3/50, 3/50 and 12/50 for females of the control, low-, mid- and high-dose groups, respectively. Female rats in all treated groups showed a dose-related increase in the incidence of adrenal cortical hyperplasia (16/49, 26/50, 27/50 and 31/50 for control, low-, mid- and high-dose groups, respectively). In male rats of the mid- and high-dose groups, an increased incidence of testicular atrophy was found (10/50, 10/49, 33/50 and 49/49 for control, low-, mid- and high-dose groups, respectively). The incidence of mammary neoplasms (unspecified) increased in all treated females, but without a dose–response relationship (29/49, 41/50, 45/50 and 40/50 for control, low-, mid- and high-dose groups, respectively). The combined incidence of mammary adenocarcinoma and carcinosarcomas in female rats was 1/49, 1/50, 3/50 and 8/50 for control, low-, mid- and high-dose groups, respectively. In male rats, the incidence of dermal fibroma increased in all treatment groups, but without a dose–response relationship. Sebaceous gland adenoma (6/49) and adenocarcinomas (1/49) were found in only high-dose males. In males of the mid- and high-dose groups, the incidence of neural astrocytomas increased (2/50 and 5/49, respectively), whereas none of these tumours was found in the control or low-dose group (King et al., 1972b; Halliday et al., 1973b).

No chronic toxicity studies were identified for **AOZ**.

For **furaltadone**, only one carcinogenicity study was identified in the scientific literature available in the public domain. In another study that had been submitted by industry to JECFA for the evaluation of nitrofurazone (FAO/WHO, 1993b), furaltadone was also applied. This study (Siedler and Sierfoss, 1966; see below) was made available to EFSA by the data owner.

Furaltadone hydrochloride (‘pure’) was administered to a group of 36 weanling female Sprague–Dawley rats, weighing 40–72 g, in the diet at a concentration of 1 g/kg diet for a period of 46 weeks. The animals were maintained on a control diet for an additional period of 20 weeks. A group of 26 untreated females served as the control group. Using the reported information on cumulative dose, duration of administration, the growth of the animals and a conversion for the molecular weight of furaltadone hydrochloride to furaltadone, the average furaltadone dose was estimated by the

CONTAM Panel to be 54 mg/kg b.w. per day. A significant increase ($p < 0.001$) in the incidence of mammary adenocarcinomas (25/32 versus 0/25 in the control) was observed. Based on this observation, the authors concluded that furaltadone was strongly carcinogenic (Cohen et al., 1973).

Groups of 35 female Holtzman rats were fed diets containing 0 or 0.15 % furaltadone (purity not given), corresponding to reported average doses of 0 or 85 mg/kg b.w. per day, respectively, for 45 weeks, followed by control diet for 8 weeks. At termination (week 53), survival was reduced; 25/35 versus 33/35 in the control group. In treated animals, the incidence of mammary adenomas was 22/35 versus 2/35 in the controls, and the incidence of adenocarcinomas was 3/35 compared with none in the controls (Siedler and Searfoss, 1966).

No chronic toxicity studies were identified for **AMOZ**.

For **nitrofurantoin**, several carcinogenicity studies were identified in the scientific literature available in the public domain.

Nitrofurantoin (purity and crystalline form not specified) was administered to groups of 52–53 male and 54 female BDF1 mice at 0, 750 or 3 000 mg/kg of diet. These dietary concentrations correspond to doses of 0, 112.5 or 450 mg/kg b.w. per day, respectively, using the default factor of 0.15 as recommended by the EFSA SC (2012) for a 2-year mice study. At the end of the experiment, survival in males and females combined was 50.5, 42.5 and 46.2 in control, low-dose and high-dose groups, respectively. In males, a reduced incidence of hepatic adenomas was observed; 6/53 in controls, 1/52 in low-dose mice and 0/52 in high-dose mice. No increase in the incidence of tumours at any site was observed (Ito et al., 1983).

Groups of 50 male and 50 female Swiss (Crl:CDR_1(ICR)BR) mice, about 50 days of age, received nitrofurantoin (pharmaceutical grade macrocrystals) in the diet for 22 months at concentrations equivalent to an average dose of 0, 50, 100 or 200 mg/kg b.w. per day (0, 44, 84 or 181 mg/kg b.w. per day for males and 0, 59, 116 or 224 mg/kg b.w. per day for females, respectively). Increased mortality was observed in high-dose males. Kidney effects such as tubular dilatation and hyperplasia were found in males and females of the mid- and high-dose groups. In males, the incidence of malignant lymphomas at all sites were 2/50 (controls), 6/50 (low dose), 4/49 (mid dose) and 10/50 (high dose), reaching significance at the high dose ($p = 0.012$) only. However, the authors suggested that the increase in this 'common neoplasm' was not related to treatment (Butler et al., 1990b).

Groups of 50 male and 50 female B6C3F1 mice, 8 to 9 weeks of age, were fed nitrofurantoin (pharmaceutical grade microcrystalline powder) for 103 weeks at 0, 1 300 or 2 500 mg/kg diet. Calculated average doses were reported to be 0, 277 or 577 mg/kg b.w. per day for females and 0, 295 or 567 for males, respectively. Mortality at termination of the experiment was reduced in females; survival rates were 19/50, 37/50 and 37/50 in control, low-dose and high-dose groups, respectively. In females, ovarian atrophy was seen in treated mice (48/50 in low-dose and 49/50 in high-dose animals), compared with no such effect in the controls. In high-dose mice, kidney effects (mineralisation of the renal medulla in females, and dilatation of renal tubules in males) were observed. High-dose males showed testicular degeneration. In female mice, an increased incidence of malignant lymphomas was observed: 12/50, 19/50 and 24/50 in control, low-dose and high-dose animals, respectively. Because of unusually low survival of female mice in the control group (animals started to die in week 65 compared with in about week 85 for both dose groups; mortality at the end of the study was 60 % for the control group versus about 25 % for treated females), NTP calculated adjusted rates for these malignant lymphomas: 50.2 % (control), 43.4 % (low dose) and 52.7 % (high dose). These results indicate that there is no treatment-related increase in malignant lymphomas in female mice. In high-dose male mice, survival was only slightly higher (about 10 %) than that in the control and low-dose groups, and no increase in any type of malignant tumours was observed (NTP, 1989).

In a study on ovarian atrophy, three groups of female B6C3F1 mice ($n = 20$), 5 to 6 weeks of age, were administered nitrofurantoin (pharmaceutical grade) in the diet at concentrations corresponding to

doses of 0, 350 or 500 mg/kg b.w. per day, respectively, for 64 weeks. At termination, survival was 20 in controls, 19 in low-dose and 18 in high-dose animals. At the end of the study, ovarian atrophy was found in only treated animals: 0/20 in control, 18/19 in low-dose and 18/18 in high-dose animals. There was no increase in the incidence of neoplasms of the reproductive system (ovaries, uterus, vagina), which were the only tissues examined (Stitzel et al., 1989).

A group of 10 pregnant ICR/Jcl mice received three s.c. injections of nitrofurantoin (purity unspecified) at 75 mg/kg b.w. suspended in a 1 % gelatine solution on days 13, 15 and 17 of gestation. Groups of 22 gelatine-treated dams and 76 untreated dams served as controls. Offspring were foster-nursed by untreated dams and were sacrificed 32 weeks after birth. Gross pathological lesions were examined for tumours. The incidence of papillary adenomas of the lung increased in the offspring of nitrofurantoin-treated dams (10/78 (12.8 %), $p < 0.002$) compared with gelatine-treated controls (5/203 (2.5 %)) and untreated controls (29/478 (5.3 %)) (Nomura et al., 1984).

A group of 36 weanling female Sprague–Dawley rats, weighing 40–72 g, was administered nitrofurantoin ('pure') at 1 870 mg/kg diet for 16 weeks. Because of impaired growth and premature mortality, the dose was reduced to 100 mg/kg diet for week 16–75. From week 75 to 80, the animals were kept on a control diet. Using the reported information on the cumulative dose, duration of administration and growth of the animals, the average dose on a body weight basis was estimated to be 187 mg/kg b.w. per day for the first 16 weeks and about 7 mg/kg b.w. per day for the second period. The experiment was terminated at week 80. A group of 30 untreated rats served as controls. No increase in tumour incidence was observed (Cohen et al., 1973).

Two groups of weanling, germ-free female Sprague–Dawley rats (11 control and 12 treated rats), weighing 85–100 g, were fed nitrofurantoin (extracted from pharmaceutical grade, macrocrystalline nitrofurantoin) at 0 or 1 880 mg/kg of diet for 104 weeks. Using the default factor of 0.05 as recommended by the EFSA SC (2012) for a 2-year rat study, this dietary concentration corresponds to a dose of 94 mg/kg b.w. per day. The growth rate in treated rats was slightly retarded compared with controls. The median survival time was 96 weeks for controls and 90 weeks for treated animals. The incidences of mammary fibroadenomas were 2/11 in controls and 9/12 in rats treated with nitrofurantoin ($p < 0.01$, Fisher's exact test). No increase in the incidence of tumours at other sites was observed (Wang et al., 1984).

Groups of Fischer 344 rats (50/sex/group) were administered nitrofurantoin (pharmaceutical grade microcrystalline powder) in the diet containing 0, 600 or 1 300 mg/kg (females) and 0, 1 300 or 2 500 mg/kg (males) for 103 weeks. Calculated average doses were reported to be 0, 28 or 62 mg/kg b.w. per day for females and 0, 59 or 111 mg/kg b.w. per day for males, respectively. Survival at termination of the experiment did not differ between control and treated animals. Chronic tubular nephropathy was observed in all treated rats, but the authors judged the severity to be greater in dosed males. The incidence of microscopic renal tubular adenomas was 3/50 in controls, 11/50 in low-dose males and 19/50 in high-dose males. In high-dose male rats, testis degeneration and atypical cells of the epididymis were observed. Renal tubular carcinomas were seen in two high-dose males. Osteosarcomas were seen in one low-dose male and two high-dose males. Reductions in the incidences of preputial gland adenomas and carcinomas, and interstitial cell adenomas of the testes were observed in males. No change in tumour incidence was observed in female rats (NTP, 1989).

Butler et al. (1990a) administered groups of 60 male and female Sprague–Dawley rats nitrofurantoin (pharmaceutical grade macrocrystals) in the diet for 24 months at concentrations equivalent to an average dose of 0, 24, 48 or 96 mg/kg b.w. per day. Body weight gain was reduced in high-dose females and mortality increased in high-dose males. In high-dose males, an increased incidence of testicular degeneration and epididymal fibrosis was observed. In the same paper (Butler et al., 1990a), a separate oncogenicity study was reported, in which groups of 50 male and female Sprague–Dawley rats received nitrofurantoin in the diet for 24 months at concentrations equivalent to 0, 12, 24 or 48 mg/kg b.w. per day. No effects on mortality were found and no increased incidence in neoplasms at any site was observed.

No chronic toxicity studies were identified for **AHD**.

Several carcinogenicity studies on **nitrofurazone** were identified in the scientific literature available in the public domain. In addition, the CONTAM Panel identified two carcinogenicity studies on nitrofurazone (Siedler and Searfoss, 1966, 1976) that had been submitted by industry to JECFA for the evaluation of nitrofurazone (FAO/WHO, 1993b). The original documents were made available to the CONTAM Panel by the data owner.

Nitrofurazone (99 % pure) was administered in the diet to groups of 50 male and 50 female B6C3F1 mice at concentrations of 0, 150 or 310 mg nitrofurazone/kg, equal to 0, 14 or 29 mg/kg b.w. per day, respectively, for 2 years. At the end of the experiment, survival was reduced, particularly in male mice: 31/50 and 27/50 for the low- and high-dose groups, respectively, compared with 39/50 for controls. In female mice, there was an increased incidence of ovarian atrophy: 7/47 in controls, 44/50 in the low-dose group and 38/50 in the high-dose group. The incidence of ovarian granulosa cell tumours increased: 4/50 and 9/50 in low- and high-dose females, compared with 1/47 in control animals. In addition, the incidence of benign mixed tumours of the ovary increased: 17/50 in the low-dose group and 20/50 in the high-dose group, compared with 0/47 in the controls. In male mice, there were no increased incidences of any tumour type (NTP, 1988; Kari et al., 1989).

In a study on transplacental carcinogenesis reported by Nomura et al. (1984), a group of 20 pregnant ICR/Jcl mice received three s.c. injections of nitrofurazone (purity not specified) at 75 mg/kg b.w. suspended in 1 % gelatine solution on days 13, 15 and 17 of gestation. The offspring were foster-nursed by untreated dams. Gross pathological lesions were examined for tumours. In contrast to nitrofurantoin (see above), no increase in lung tumours (papillary adenomas) was observed when the offspring were examined after 32 weeks. In the same study, new-born mice (n = 17) received one s.c. injection of 75 mg nitrofurazone/kg b.w. 12 hours after birth and three further injections on days 7, 14 and 21. After 32 weeks, the incidence of papillary adenomas of the lung had increased: 19.7 % in the treated animals compared with 2.5 % in the controls.

Groups of 35 female Holtzman rats were fed diets containing 0, 0.05 or 0.1 % nitrofurazone (purity not given), corresponding to reported average doses of 0, 28 or 55 mg/kg b.w. per day, respectively, for 45 weeks, followed by a control diet for 8 weeks. At termination (week 53), survival was reduced in the high-dose group (23/35) compared with both the control and the low-dose group (both 33/35), and the incidence of animals with benign mammary tumours (adenomas or adenofibromas) increased: 10/35 in the low-dose group and 12/35 in the high-dose group, versus 2/35 in the controls (Siedler and Searfoss, 1966).

Male and female CFE rats (20/group/sex) were fed diets containing nitrofurazone corresponding to a daily intake of 55 mg/kg b.w. per day for females and 50 mg/kg b.w. per day for males for 45 weeks. At the end of this period, the animals were maintained on a control diet for a further 7 weeks. There was no increased incidence of any tumour type in male rats. In females, a significant increase in the incidence of benign mammary tumours was noted at week 52 (12/20) compared with untreated controls (0/20) (Siedler and Searfoss, 1967).

In two experiments, nitrofurazone (purity about 97 %) was administered in the diet at a concentration of 0.1 %, corresponding to 150 mg/kg b.w. per day, to a group of 20 female Holtzman rats for 36 weeks (experiment 1) and 30 female rats for 44.5 weeks (experiment 2). Animals were killed 15–19 weeks after dosing was completed. At termination, the incidence of mammary fibroadenomas in rats fed nitrofurazone-containing diets increased (11/18 in experiment 1 and 24/24 in experiment 2) compared with the controls, for which there was none (Morris et al., 1969).

A group of 30 female weanling Sprague–Dawley rats were administered ‘pure’ nitrofurazone at 100 mg/kg diet for 46 weeks (average daily intake was 8–13 mg/rat). After the dosing period, the animals were maintained on a control diet for 20 weeks. A control group of 30 rats received a control

diet for 66 weeks. The incidence of mammary fibroadenomas in treated females that lived 22 weeks or more was 22/29, compared with 2/29 in the control group (Ertürk et al., 1970).

Groups of 50 male and 50 female Fischer 344/N rats, 6 to 7 weeks of age, were administered nitrofurazone (99 % pure) at 0, 310 or 620 mg/kg diet for 103 weeks. The average amount of nitrofurazone consumed was 11–12 mg/kg b.w. per day for low-dose and 24–26 mg/kg b.w. per day for high-dose animals. Surviving animals were killed at week 111. At the end of the experiment, survival was reduced in high-dose males (20/50) compared with the control (33/50) and low-dose group (30/50). In male rats, increased incidences of testicular degeneration, characterised by atrophy of the germinal epithelium and aspermatogenesis, were observed (12/50, 49/50 and 47/50 for control, low-dose and high-dose groups, respectively). Adenomas of the sebaceous glands were observed in high-dose males only (4/50). The incidence of mammary fibroadenomas increased without a dose–response relationship in treated females: 8/49 (control), 36/50 (low dose) and 36/50 (high dose). In one control and two high-dose females, adenocarcinomas were observed. In males, the incidence of testicular interstitial cell tumours decreased with dose (45/50 in controls, 30/50 in low-dose group and 28/50 in high-dose group), whereas the incidence of carcinoma of the preputial gland increased with dose (1/50 in controls, 8/50 in low-dose group and 5/50 in high-dose group), but without a dose–response relationship. However, the combined incidence of preputial gland adenomas and carcinomas was not statistically different from the controls. The authors stated that this combined incidence is the most appropriate value to be used in the evaluation of these neoplasms because the adenomas and carcinomas are derived from the same cell type, they form a morphological continuum and distinction between both types of tumours is difficult to make (NTP, 1988; Kari et al., 1989).

In a study by Mori et al. (1960) evaluating the induction of pulmonary tumours, female mice (group size not given) were fed a diet containing 0.1 % **SEM hydrochloride** for 7 months (equivalent to approximately 150 mg/kg b.w. per day). At termination of the study, six of the eight survivors (75 %) had developed lung tumours (not specified), compared with 1 of the 20 (5 %) control animals.

In a limited study, Toth et al. (1975) tested the effect of SEM on the development of lung and blood vessel tumours in Swiss mice. SEM dissolved in drinking water at a concentration of 0.0625 % was given to 50 male and 50 female mice for their lifetime. Groups of 100 male and 100 female mice served as controls. Using a conversion factor of 0.09 for a chronic drinking water study (EFSA SC, 2012), this corresponds to a dose of 56 mg/kg b.w. per day. The incidence of lung tumours (adenomas and adenocarcinomas) increased in treated males (30 % compared with 23 % in the controls) and females (50 % compared with 21 % in the controls). Of these tumours, 20 % were reported to be adenocarcinomas. The incidence of blood vessel tumours increased in female mice (18 % compared with 5 % in the controls), but not in males.

Groups of male and female Charles River CD rats (26 per sex) were administered a diet containing 0, 500 or 1 000 mg SEM hydrochloride for an intended period of 104 weeks (Weisburger et al., 1981). Using a default factor of 0.05, as recommended by the EFSA SC (2012) for a 2-year rat study, the dietary concentrations correspond to a dose of 0, 25 or 50 mg SEM hydrochloride/kg b.w. per day, respectively. Mortality in the high dose was so large that treatment was discontinued at week 32 and the animals were maintained on a control diet. For the low dose, the treatment was stopped at week 78. Signs of gross toxicity such as protrusion of the sternum, bowing of the legs and stiffness of the joints were observed. Upon histological examination, osteoporosis was seen in the long bones. No numerical details for effects in the different dose groups were reported. There was no indication of tumour induction.

To study the chronic toxicity of SEM hydrochloride, Takahashi et al. (2014) fed groups of Wistar Hannover GALAS rats a diet containing 0, 10, 50 or 250 mg/kg SEM hydrochloride (purity 99.3 %) for 52 weeks. The dietary concentrations corresponded to reported average doses of 0, 0.6, 3.5 or 16.7 mg/kg b.w. per day for males and 0, 0.8, 4.5 or 21.8 mg/kg b.w. per day for females, respectively. In addition, four groups of Wistar Hannover GALAS rats (50/sex/group) were fed a similar diet for 104 weeks, corresponding to reported average doses of 0, 0.6, 3.2 or 14.8 mg/kg b.w. per day for

males and 0, 0.8, 3.8 or 19.4 mg/kg b.w. per day for females, respectively, to investigate the carcinogenicity of SEM hydrochloride. No effect on survival was found in the 1-year study, but mortality increased in mid- and high-dose males (8/50, 9/50, 17/50 and 15/50 for control, low-, mid- and high-dose groups, respectively) and in mid- and high-dose females (7/50, 10/50, 17/50 and 13/50 for control, low-, mid- and high-dose groups, respectively). In the 1-year study, haematological parameters and organ weights were not affected by treatment, but chlorine and glucose levels in serum significantly increased in high-dose males and females. Upon histopathological examination, effects on bones (disarrangement of chondrocytes) and joints (degeneration of articular cartilage) were found in mid- and high-dose males and females, and males appeared to be more sensitive than females. In mid- and high-dose males, the incidence of disarrangement of chondrocytes accompanied with an increase in connective tissue in the tibia and sternum was 5/10 and 10/10 and in vertebrae was 2/10 and 8/10, respectively. These effects were not observed in the controls or low-dose animals. In addition, the observed degeneration of the articular cartilage of the knee joints found in both males and females was more prominent in males (2/10 and 10/10 for mid- and high-dose groups, respectively, versus 0/10 in both the controls and low-dose group). These effects on bones were also found in the 2-year study at comparable incidences. In this study, no effect on the incidence of any type of tumour, including lung tumours, was observed for male and female rats. Based on the effects on bones, a NOAEL of 0.6 mg/kg b.w. per day can be derived.

The CONTAM Panel identified two carcinogenicity studies for **nifursol** that had been submitted to SCAN for its evaluation as a feed additive (SCAN, 2001, 2003). The original documents were made available by the data owner. No carcinogenicity studies on nifursol were identified in the public literature.

Groups of Simonsen Long–Evans rats (40 per sex) were fed nifursol at dietary concentrations of 0, 400, 600, 800 or 1 000 mg/kg for 2 years and 3 months (Rude, 1970c). Using the default factor of 0.05 as recommended by the EFSA SC (2012) for a 2-year rat study, these dietary concentrations correspond to 0, 20, 30, 40 or 50 mg/kg b.w. per day, respectively. The study was terminated when mortality reached 30 %: 117 weeks for males and 118 weeks for females. Survival at the end of the study was reduced in males of the two highest dose groups (70 and 75 % versus 92.5 % in the controls). No statistically significant differences in body weight gain or food intake were reported between controls and treated groups, and no treatment-related effects were seen following haematological or urine analysis. However, no clinical chemistry parameters were measured in this study. No increase in organ weights was observed, but liver weights showed a statistically significant decrease in a dose-related manner at 30 mg/kg b.w. per day and higher in males. No dose-related effect on liver weight was found in females. Histopathological examination did not reveal any treatment-related effect. Some common benign neoplasms, such as interstitial cell adenoma in males, mammary fibroadenomas in females and subcutaneous fibroma in both males and females, were found, but differences did not show a dose–response relationship and were not statistically significant. Dawe (1988), presenting a separate statistical analysis of the neoplastic changes observed in this study, confirmed a positive trend for renal tubular adenoma in male rats (0/25, 1/20, 1/20, 1/25 and 3/25 for control, 20, 30, 40 and 50 mg/kg b.w. per day, respectively), but concluded that the difference between the control and the high-dose group was not significant (Fisher exact probability test, $p > 0.1$). The incidence of mammary adenocarcinoma in female rats was not increased. Therefore, the authors concluded that nifursol had no carcinogenic effect when given to rats in their feed for a period of 2 years and 3 months. The CONTAM Panel concluded that the study design was too limited to derive a NOAEL for non-neoplastic effects.

Five groups of four male and four female Beagle dogs were fed diets containing 0, 400, 600, 800 or 1 000 mg/kg nifursol for 2 years. Using the default factor of 0.25 for a chronic dog study, these dietary concentrations correspond to 0, 10, 15, 25 or 50 mg/kg b.w. per day, respectively. Tissue from animals with gross pathology and liver tissues from all dogs were examined microscopically. Liver effects such as mononuclear cell infiltration, dark pigmented Kupffer cells and periportal infiltration were found in males in a dose-related manner at 15 mg/kg b.w. per day and higher. Similar effects were seen in all female dogs (including controls), but without a dose–response relationship. Abnormalities

of the gall-bladder (e.g. dilatation and amorphous material in the lumen) were found in high-dose males and in females of the two highest dose groups. The authors stated that the liver lesions were difficult to evaluate owing to infection with heart worm (*Dirofilaria immitis*) in a number of the dogs. No neoplasms were observed in this study.

No chronic toxicity studies were identified for **DNSH**.

Conclusions

Furazolidone induced malignant tumours in mice and rats. These included mammary tumours in rats, bronchial adenocarcinomas in male and female mice and neural astrocytomas in male rats. Based on the increased incidence of these malignant tumours, the CONTAM Panel concluded that furazolidone is carcinogenic. JECFA (FAO/WHO, 1993a) concluded that furazolidone is a genotoxic carcinogen. Although the **AOZ** side-chain, which may be released from the parent compound and from bound residues, may play a role in tumour formation, no experimental information is available on AOZ.

Furaltadone induced malignant mammary tumours in female rats. No studies in mice were identified. Although the CONTAM Panel noted the limitations of the two available studies (small number of animals, only female rats tested, only one dose level and short dosing period of 46 weeks), it concluded that furaltadone is carcinogenic to rats. In addition, IARC (1987) concluded that there was sufficient evidence for its carcinogenicity in experimental animals and classified furaltadone in Group 2B (possibly carcinogenic to humans).

No chronic toxicity studies were identified for **AMOZ**.

Nitrofurantoin was tested for its carcinogenicity in several studies in mice and rats and was found to induce predominantly an increase in benign tumours (e.g. ovarian tubular adenomas, mammary fibroadenomas). In one mice study, an increase in malignant lymphomas in males was observed, but the authors concluded that the increase of this common tumour in mice was not treatment related. Adjusted rates for the incidence of malignant lymphomas in male B6C3F1 mice as reported in the NTP study (NTP, 1989) did not show any difference between control and treated mice. In male rats, a few malignant tumours were found (renal tubular carcinomas in two high-dose males and osteosarcomas in one low-dose male and two high-dose males). Based on these observations, the CONTAM Panel concluded that there is limited evidence that nitrofurantoin is carcinogenic in rats. IARC (1990a) concluded that two of the four studies in mice, including the transplacental study, were inadequate for the evaluation. In one study, the incidence of ovarian tubular adenomas and benign mixed tumours increased in female mice, and in another study there was an increase in malignant lymphomas in male mice. In one rat study, an increase in mammary fibroadenomas was observed. IARC (1990a) concluded that there was limited evidence for the carcinogenicity of nitrofurantoin in experimental animals, and classified it in Group 3 (not classifiable as regards its carcinogenicity in humans).

No chronic toxicity studies were identified for **AHD**.

Nitrofurazone increased the incidence primarily of benign tumours in mice and rats following oral administration. In mice, an increase in the incidence of granulosa cell tumours and benign mixed tumours of the ovary was observed and, in rats, an increase in mammary fibroadenomas was observed. In male rats, a non-dose-related increase in carcinomas of the preputial gland was observed, but the combined incidence of preputial gland adenomas and carcinomas, which was considered to be the most appropriate parameter for this type of tumour, was not affected. The CONTAM Panel concluded that there is no evidence for the carcinogenicity of nitrofurazone in mice, and that there is equivocal evidence for its carcinogenicity in rats. Non-neoplastic effects were observed at the lowest dose (14 mg/kg b.w. per day) tested in mice (reduced survival in males and ovarian atrophy) and the lowest dose (11–12 mg/kg b.w. per day) tested in rats (testis degeneration), precluding the derivation of a NOAEL. JECFA (FAO/WHO, 1993b) concluded that the data suggest that nitrofurazone is a

secondary carcinogen, and that effects on steroidogenesis may be involved in the process of tumour formation. IARC concluded that there is limited evidence for the carcinogenicity of nitrofurazone in experimental animals and inadequate evidence for its carcinogenicity in humans. In its overall evaluation, IARC placed nitrofurazone in Group 3 (not classifiable as regards its carcinogenicity in humans) (IARC, 1990b).

SEM was reported to increase the incidence of lung tumours in two limited studies in mice. In one study, the lung tumours were not specified; in the other study, an increase in malignant lung tumours is indicated, particularly in female animals. In the two available rat studies, including a recent and well-conducted study, no increase in tumour incidence was found. Recognising the shortcomings of most of these studies, the CONTAM Panel concluded that there is limited evidence that SEM is carcinogenic in mice, but not in rats. In addition, IARC (1987) evaluated SEM and concluded that there was inadequate evidence for carcinogenicity of SEM in humans and inadequate or limited evidence (lung tumours in mice) in experimental animals, and classified it as Group 3 (not classifiable as regards its carcinogenicity in humans). The AFC Panel (EFSA, 2005) noted that SEM had been shown to be carcinogenic in mice but not in rats and concluded that SEM is a weak non-genotoxic carcinogen for which a threshold mechanism can be assumed. Based on the effects on bones observed in male rats, a NOAEL of 0.6 mg/kg per day can be derived.

Based on the available information on **nifursol**, the CONTAM Panel concluded that there is no clear indication of carcinogenic activity of nifursol. Because the available information was too limited, the CONTAM Panel was not able to derive a NOAEL for nifursol.

No chronic toxicity studies were identified for **DNSH**.

8.3. Modes of action

Nitrofurans have been used as antibacterial agents and to some extent still are. They are rapidly metabolised. The reduction of the nitro-group, the primary biotransformation route, initially results in the formation of a radical nitroanion. The oxidation of this radical by oxygen back to the parent compound results in the formation of superoxide and other radical oxygen species. This may lead to oxidative stress and may be responsible for some of the adverse effects observed at higher levels of the drugs, including some of the adverse effects in genotoxicity tests. Furazolidone (Jin et al., 2011), but also AOZ and AMOZ (Zolla et al., 2005) and SEM (Hirakawa et al., 2003) caused DNA damage and DNA fragmentation in *in vitro* systems by the production of ROS, which could be ameliorated by addition of catalase or superoxide dismutase. These oxygen radicals can be detoxified by GSH resulting in the formation of the oxidised form, GSSG, and potentially increased synthesis and even depletion of GSH levels in the cell (Hoogenboom et al., 1992b). Antioxidants such as vitamin E and selenium may also protect against damage (Boyd et al., 1979; Peterson et al., 1982), whereas polyunsaturated fatty acids, for example, may exaggerate the effects (Boyd et al., 1979).

Further reduction of the nitro-group is thought to lead to a number of other highly reactive metabolites such as the hydroxylamine and acrylonitrile derivatives. *In vitro* studies revealed that the latter can bind to compounds containing thiol groups such as GSH or mercaptoethanol, forming a rather unstable adduct (Vroomen et al., 1987b, 1990). Incubation of the GSH adduct with proteins results in a switch of the nitrofuran adduct to the protein. Incubation of that protein with GSH results in the reversed reaction. This process is called a retro-Michael reaction. However, it is unclear to what extent the binding of the acrylonitrile derivative to proteins is responsible for the protein adducts observed both *in vitro* and *in vivo*, i.e. the adducts initially detected *in vivo* and *in vitro* by the use of radiolabelled drugs (Vroomen et al., 1986, 1987c; Hoogenboom et al., 1991c). Incubation of such adducts in pig liver or pig hepatocytes with mercaptoethanol did not result in the exchange of the nitrofuran part. It might be that the acrylonitrile derivative can also bind to other non-thiol amino acids, thereby forming more stable adducts.

The formation of protein adducts may be involved in certain adverse effects of nitrofurans such as the irreversible inhibition of the pyruvate dehydrogenase complex, which is responsible for the conversion of pyruvate into acetyl-CoA, which is essential in the case of nerve cells (Paul et al., 1952, 1953, 1956). In pig hepatocytes, this resulted in the accumulation of lactate in the culture medium, an effect from which the cells only slowly recovered (Hoogenboom et al., 1991a). In human patients treated with furazolidone, increased levels of pyruvate and lactate were observed in plasma (Paul and Paul, 1964). This inhibition is likely to be the cause of the polyneuritis observed in patients treated with the drugs. It is unclear if other enzymes are affected by the formation of adducts, although reduced activity of GSH reductase has also been reported (Murakami et al., 1989).

The reactive intermediates of nitrofurans may also be able to form DNA adducts and are most likely responsible for mutagenic effects observed in bacterial tests at rather low levels (see Section 8.2.6). A similar mechanism may cause the genotoxic effects observed in mammalian cells, although oxidative stress may also play a role. Hiraku et al. (2004) reported that nitrofurazone could be metabolised by CYP to produce short-lived nitro-radicals and cause formation of 8-oxodG DNA adducts in isolated calf thymus DNA, as well as in exposed human promyelocytic leukaemia cells HL-60. Recently Jin et al. (2011), in an *in vitro* study with metabolically active human hepatoma cells (HepG2), showed that exposure to furazolidone induced increased formation of intracellular ROS and formation of 8-hydroxydeoxyguanosine, associated with damage to nuclear and mitochondrial DNA and cell cycle arrest. *In vivo* in F344 gpt delta rats nitrofurantoin at carcinogenic dose induced an increase in mutation frequency and eleveated elevels of 8-oxodG DNA adducts in the kidney (Kijima et al. 2015). The predominant mutations were G-base substitutions and considering that 8- oxodG causes G:C-T:A transversion mutations (Cheng et al., 1992), oxidative DNA damage including 8- oxodG formation might contribute to guanine base substitution mutations observed in the gpt gene following nitrofurantoin exposure. In rats and mice, most nitrofurans have been shown to be carcinogenic (see Section 8.2.7). As a result, a number of these drugs are classified as genotoxic carcinogens.

The nitrofurans discussed in this opinion consist of a nitrofuran ring connected to a side-chain via an azomethine (C=N) bond. Under acid conditions, this bond is rather labile and this can result in the release of the free side-chain. Using the weakness of the azomethine bond at low pH, a method was developed for the detection of protein adducts (bound residues) based on the release of the side-chain from the proteins, subsequent derivatisation with NBA and detection by LC-UV and later LC-MS (Hoogenboom et al., 1991c). Using this method, it was confirmed that these protein-bound residues should be regarded as drug-like adducts and are not the result of incorporation of small radiolabelled fragments of the parent compound into amino acids and proteins. Although levels decrease over time, these adducts can be detected in treated animals for many months (see Section 8.1). As shown by Liu et al. (2010a), this gradual degradation of bound residues allows the detection of the AOZ side-chain in urine for many weeks.

The free side-chains all contain an H_2N-N part and are as such hydrazines, which in general are suspected carcinogens. Release of these side-chains may occur in the stomach and they may be regarded as metabolites. However, detection of the free side-chains requires a special analytical procedure and has rarely been studied. Free AOZ was detected in the blood of pigs treated with AOZ, but also with furazolidone, the latter confirming that the release and absorption of free side-chains in the stomach is feasible (Hoogenboom et al., 2002). This study indicated that a substantial part, but not all, of the furazolidone was hydrolysed to AOZ. Furthermore, AOZ levels in plasma decreased only slowly and were similar in the hepatic portal vein and mesenteric artery, indicating slow degradation and excretion. It was also shown that free AOZ could be detected in the blood of rats fed with meat from furazolidone-treated pigs. This implied that consumers could be exposed to free AOZ, requiring more information on its properties.

Early studies hypothesised that AOZ, released in the stomach, actually plays an important role in the neurotoxic effects of furazolidone (Palm et al., 1967; Stern et al., 1967). In particular, the inhibition of MAO, an enzyme involved in detoxification of amines such as tryptamine, could play a role. Initially it was hypothesised that ring cleavage of AOZ would result in the formation of HEH and that HEH

was responsible for the inhibition. *In vitro* studies revealed that both AOZ and HEH could irreversibly inhibit MAO activity (Hoogenboom et al., 1991b), but there is no proof for the conversion of AOZ into HEH. In this study, nitrofurans were found to be able to inhibit MAO in cultured pig hepatocytes but in a more reversible way.

Incubation of pig hepatocytes with AOZ resulted in the formation of protein-bound metabolites, initially detected by the use of a radiolabelled compound (Hoogenboom et al., 2002). This formation could be inhibited by both dimethyl sulphoxide and 4-chlorobenzenesulphonamide (a known inhibitor of amidohydrolases), a strong indication that AOZ is metabolised into a reactive metabolite. Both compounds also reduced the MAO inhibition by AOZ in pig hepatocytes, suggesting that the binding of this reactive metabolite is also responsible for this effect. Remarkably, AOZ could be released from these protein adducts under acid conditions and detected after derivatisation with NBA. This could explain the observation that AOZ can be released from tissues of rats fed with meat containing bound residues of furazolidone (McCracken et al., 1997). Furthermore, it was shown that AOZ is a mutagenic compound in bacteria and causes genotoxic effects in mammalian cells (Hoogenboom et al., 2002), potentially because of the formation of DNA adducts. As AOZ is likely to also be a metabolite of furazolidone in rats and mice, it cannot be excluded that it plays an important role in the carcinogenic effects observed with the drug.

An important question is to what extent other nitrofurans differ from furazolidone, owing to the presence of different side-chains. It is clear from both *in vitro* and *in vivo* studies that furaltadone, nitrofurantoin, nitrofurazone and nifursol are also able to form protein adducts from which the side-chain can be released under acid conditions (see Sections 8.1.4 and 8.1.5). However, only for furazolidone was it shown that the side-chain was actually released from the parent compound or the protein-bound residues and can as such be considered as a metabolite. For the other compounds, no studies were found that investigated this.

The limited studies in rats show a similar potency for furaltadone for inducing mammary adenocarcinomas as for furazolidone (see Section 8.2.7). Furaltadone was also positive in mutagenicity tests with bacteria and mammalian cells (see Section 8.2.6). However, contrary to the finding for AOZ, AMOZ tested negative in the *Salmonella*/microsome test (NOTOX, 1994e) and was also not clastogenic in the *in vitro* chromosomal aberration assay with peripheral human lymphocytes both with and without metabolic activation (NOTOX, 1994c). When tested on pig hepatocytes, AMOZ showed an inhibition of MAO activity, but at much higher concentrations than AOZ (Hoogenboom et al., 1994).

Nitrofurazone showed induction of benign tumours in animals but apparently no malignant tumours. There is limited evidence for the induction of lung tumours in mice by SEM, an effect not observed with nitrofurazone, although an increased incidence of papillary adenomas was reported (see Section 8.2.7). SEM showed clear mutagenic effects in bacteria and is genotoxic in some but not all tests with mammalian cells (see Section 8.2.6). Furthermore, SEM caused increased formation of micronuclei in the bone marrow of treated rats (Vlastos et al., 2010), although a clear dose–response curve was lacking.

The CONTAM Panel noted that the observed effects in rats and mice also point to other mechanisms that are not genotoxic but may, for example, lead to the formation of mammary tumours, such as a disturbance of hormonal balance. For nitrofurazone, for example, the long-term treatment resulted in an increased incidence of ovarian atrophy, benign mixed ovary tumours and ovarian granulosa cell tumours in female mice, and increased incidence of benign mammary tumours in rats. In male rats, an increased incidence of testicular degeneration was observed. In the case of furazolidone, an increased incidence of mammary adenomas was observed, in addition to the carcinomas. Treatment of male rats also resulted in testicular atrophy. In some of these studies, an increased incidence of adrenal cortical hyperplasia was also described, possibly resulting in a hormonal imbalance and causing some of the effects that seem to be related to this. Also *in vitro* studies provided information on an endocrine mediated mode of action. Maranghi et al. (2010) found that SEM showed weak anti-estrogenic effects

since it inhibited a number of estrogen receptor mediated activities such as the activation of hERα in transfected yeast cells, proliferation of MCF7 cells, and induction of ALP activity in an endometrial cell-line. Together with observations in juvenile rats (see Section 8.2.2) these findings brought the authors to the conclusion that SEM could act as an endocrine disruptor, possibly by intereference with estradiol (E2) signalling. However an opposite effect was found for nitrofurazone, which stimulated the proliferation of E2-dependent MCF-7 cells (Hiraku et al., 2004).

8.4. Observations in humans

8.4.1. Human pharmacological and toxicological data

In order to describe the human pharmacological and toxicological data, reviews and studies with a large number of patients ($n \geq 50$) were used, preferably those studies that provided an incidence of the adverse reactions observed. Studies reporting single or few, incidental cases have not been described.

Altamirano and Bondani (1989) have reviewed the adverse reactions to **furazolidone** that were reported in the literature between 1955 and 1989. The study included 10 443 patients treated with furazolidone of which 46 % were children, ranging in age from a few months to 15 years, and 15 % were adults. The ages of the remaining patients (39 %) were not specified. Adverse reactions to the drug were experienced by 8.3 % of the patients. These reactions were grouped as follows: gastrointestinal, neurological, systemic, dermatological and haematological reactions and adverse reactions on vital organs such as the heart, lung, liver and kidney. The most common side effects of furazolidone were gastrointestinal reactions: nausea and/or vomiting and abdominal pain. The overall frequency of the gastrointestinal reactions was 8 %. These reactions were observed for therapeutic doses of 5 to 7 mg/kg b.w. per day, although some patients received higher doses. Gastrointestinal reactions diminished if the drug was given with food. The frequency of neurological reactions to furazolidone was 1.3 %, among which the most common side effects were headache (0.72 %), vertigo (0.30 %) and giddiness (0.23 %). The frequency of systemic reactions was 0.56 % and fever was the most commonly observed side effect (0.34 %). Adverse dermatological reactions were observed in 0.54 % of the patients and skin eruptions were most common (0.38 %). Haematological side effects were found in 0.36 % of the reports and most of the adverse reactions were transient. In four reports (0.04 %), furazolidone was associated with haemolytic anaemia observed in patients deficient in G6PD. The incidence of adverse reactions on vital organs such as the heart, lung, liver and kidney were 0.11, 0.11, 0.06 and 0.06 %, respectively. Other adverse reactions have been reported, of which two are related to concomitant use of other substances. Patients who consume alcohol while taking furazolidone can develop disulfiram-like reactions. These symptoms disappeared within 24 hours after ingestion of alcohol ceased. If furazolidone is used at a dose larger than that recommended or for longer than 5 days, patients should be informed about the possibility of adverse reactions, such as a hypertensive crisis, caused by MAO-inhibitor drugs and foods containing tyramine. In the reports reviewed (n = 10 443), no deaths have been associated with adverse reactions to furazolidone. The authors concluded that most of the adverse reactions reported were mild and in only rare cases was discontinuation of the treatment necessary.

Rogers et al. (1956) reviewed laboratory and clinical data related to the use of furazolidone in animals and humans. The clinical use of furazolidone for the treatment of trichomonal vaginitis and the occurrence of adverse reactions in four study groups was reported. Furazolidone was administered in vaginal suppositories containing 0.25 % furazolidone and/or a water-soluble insufflation powder containing 0.1 % furazolidone for oral intake. Side effects of the treatments in the four study groups were briefly reported. Only one woman (n = 124) had to discontinue treatment because of local irritation. In the other patients, no systemic or local adverse drug reactions (sensitisation) were observed.

Furazolidone is used for the treatment of stomach infections caused by *Helicobacter pylori* in some developing countries in Asia and South America (see also Section 1.3.1). Zullo et al. (2012) reviewed data regarding the eradication and safety of furazolidone therapies for *H. pylori* infections. A total of

31 studies met the inclusion criteria identified in the paper and reported data of patients enrolled from 1997 to 2011. Six different furazolidone-based drug combinations have been used (including combinations with other antibiotics and bismuth salts). Furazolidone was administered twice daily at two doses, 100 or 200 mg furazolidone per dose, for 7 or 14 days. The severity of the side effects was graded as: (1) absent, (2) mild, (3) moderate (frequently interfering with daily activities), (4) marked (impeding daily activity) and (5) severe (causing treatment interruption). Side effects (grade 2 to 5) were reported by 805 (32.2 %) of 2 420 patients, who reported one or more symptoms. Severe side effects (grade 5) were reported by 3.8 % of all patients. The incidence of the side effects and severe side effects was significantly higher following the 200 mg bis in die (b.i.d., twice daily) regimen than following the 100 mg b.i.d. treatment. Similarly, the incidence of side effects and severe side effects was higher following the 14-day regimen than following the 7-day regimen. The six most reported symptoms (out of a total of 1 337 reported) were nausea (15.3 %), dizziness (13.8 %), taste disturbance (9.5 %), fatigue (9.3 %), anorexia (7.0 %) and abdominal pain (6.6 %). However, the exact composition of the six different furazolidone-based drug combinations was not reported and it is not clear how or if the side effects of the concomitant drugs could be ruled out.

Young (1961) described in a brief paper the side effects related to the i.v. administration of **furaltadone** to 54 patients suffering from miscellaneous bacterial infections. Ages of patients ranged between 6 and 71 years. Single daily doses ranged from 600 to 1 500 mg for 1 to 4 consecutive days. Multiple daily doses ranged from 200 to 400 mg per dose and were injected between one and four times per day for 1 to 7 consecutive days. The highest daily dose in the multiple dose regimen was 900 mg. Adverse reactions were observed in 5 out of 54 patients. Nausea was observed in two patients. Each of the following side effects was observed in one patient: vomiting, chills and fever after each dose, minor skin rash during therapy and urticaria during and after the last dose.

Böttiger and Westerholm (1977) have reviewed adverse drug reactions for a sulphonamide combination (sulphamethizole + sulphamethoxypyridazine) versus **nitrofurantoin** reported to the Swedish Adverse Drug Reactions Committee from 1966 to 1975. For the treatment of urinary tract infections, nitrofurantoin was given orally: 50 mg per tablet four times daily. The number of patients experiencing adverse reactions to nitrofurantoin during these 10 years was 781. Eight of these patients died (1.0 %), three of them because of interstitial pneumonia. The following adverse drug reactions were recorded: pulmonary reactions (46.4 %), fever (23.4 %), skin reactions (19.3 %), liver damage (4.6 %), blood/bone marrow reactions (2.0 %), neuropathy (2.0 %) and miscellaneous reactions (2.2 %). Remarkably, pulmonary reactions were extremely rare in the treated Swedish children. This might be because the dose in children was adjusted by many Swedish physicians to 1 mg/kg b.w. per day. The commonly used dose in adults, 50 mg four times a day, roughly corresponds to 3 mg/kg b.w. per day. Strikingly, in this review, no gastrointestinal reactions were reported. In a follow-up study from Holmberg et al. (1980), which reviewed reports of adverse reactions to nitrofurantoin received by the Swedish Adverse Drug Reaction Committee from 1966 to 1976, it was mentioned that only a few cases of gastrointestinal disturbances were reported.

Brumfitt and Hamilton-Miller (1998) reviewed the efficacy and safety profile of nitrofurantoin used for long-term prophylaxis (1 year) to prevent recurrent urinary tract infections. Between 1975 and 1992, case records were collected from 219 female patients, ranging from 9 to 89 years of age. Three dose regimens and two drug formulations were used: group A (43 patients) received 50 mg microcrystalline nitrofurantoin twice a day, group B (110 patients) received 100 mg macrocrystalline nitrofurantoin once daily and group C (66 patients) received 50 mg macrocrystalline nitrofurantoin. Patients were advised to take the medication with a snack or with milk. Side effects were observed in 37 % of all patients. Nausea was the most common adverse reaction observed for both crystalline forms. However, the incidence of nausea caused by the microcrystalline form (50 mg b.i.d.) was higher (46.5 % of group A) than the incidence of nausea observed for the macrocrystalline form used in two different dose regimens (13.6 % of group B and 12.1 % of group C). For all adverse reactions, there was a clear trend, with the lowest incidence with 50 mg of the macrocrystalline form, the intermediate incidence with 100 mg of the macrocrystalline form and the highest incidence with 50 mg twice daily of the microcrystalline form. The number of patients reporting adverse events

relating to the gastrointestinal tract, the genito-urinary tract, the skin or other reactions were 52, 13, 11 and 16, respectively. Older patients were not more likely to experience an adverse reaction than younger ones (the difference observed was not significant). It should be noted that the effective daily dose for prophylaxis is about one-quarter of that used therapeutically.

D'Arcy (1985) has analysed information retrieved from a database that was set up and maintained by the major manufacturer of nitrofurantoin (at that time), Norwich Eaton Pharmaceuticals, Inc., Norwich, New York. This database contains reported side effects available from the literature, clinical studies and reports from practitioners and regulatory authorities worldwide from 1953 to 1984. Dosage regimens of nitrofurantoin treatments have not been given. The total number of patients worldwide suffering from side effects registered in this database is 3 383. The author has calculated the incidence of adverse reactions using 121 430 000 courses of therapy as the denominator. Consequently, the overall incidence for all adverse reactions is 0.003 %. Therefore, this approach presents a very low occurrence rate for the selected side effects. The author mentions that anorexia, nausea and vomiting are the most common side effects of nitrofurantoin therapy, but these reactions are not further discussed in this paper and, consequently, these side effects are not taken into account for the calculation of the overall incidence. D'Arcy has calculated incidences for pulmonary, hepatic, neurological and haematological reactions. Incidences were very low and, of the courses of therapy, ranged from 0.001 % for pulmonary reactions to 0.0003 % for hepatic reactions. D'Arcy seperated pulmonary reactions into four categories: acute, subacute, chronic and miscellaneous pulmonary reactions. The overall fraction of patients, registered in the Norwich Eaton database, suffering from pulmonary reactions was 0.51 (1 724 out of 3 383 patients). Pulmonary reactions constituting a high proportion of the overall side effects was also observed in Swedish patients (Böttiger and Westerholm, 1977; Holmberg et al., 1980; Holmberg and Boman, 1981). Nitrofurantoin has been associated with acute hepatocellular and cholestatic injury and is less common with chronic active hepatitis. A typical neurological reaction observed during nitrofurantoin treatment was peripheral neuropathy. Referring to Holmberg et al. (1980), 2 % of all cases reported to the Swedish Adverse Drug Reaction Committee during 1966 to 1976 were attributed to polyneuropathy. A well-documented adverse reaction of nitrofurantoin is haemolytic anaemia observed in patients with G6PD deficiency.

Gleckman et al. (1979) reviewed the literature on adverse reactions to nitrofurantoin and categorised these side effects into gastrointestinal disturbances, skin eruptions, haematological disorders, neurological defects, hepatotoxicity, pulmonary complications and miscellaneous abnormalities. Incidences of the side effects were not mentioned. Anorexia, nausea and vomiting are the most common side effects, followed by skin eruptions, consisting of macular, maculopapular or urticarial lesions. These authors also mention the well-known adverse reaction haemolytic anaemia in patients with G6PD deficiency. With respect to the neurological reactions, they focus on peripheral neuropathy and mention that this side effect has also been observed for other nitrofurans such as nitrofurazone and furaltadone. Although acute and chronic hepatotoxicity has been observed, these are rare events during nitrofurantoin therapy. It is stated that nitrofurantoin-induced pulmonary reactions have been observed in hundreds of patients, but an incidence is not given. The pulmonary reactions have been classified arbitrarily into acute, subacute and chronic side effects.

Karpman and Kurzrock (2004) reviewed adverse reactions of nitrofurantoin versus those of trimethoprim and sulphamethoxazole in children. In 2004, the authors could find only two articles that specifically dealt with adverse reactions to nitrofurantoin in children (Coraggio et al., 1989; Uhari et al., 1996 - see Section 8.4.2). From a few other publications addressing adverse reactions in adults and children, incidences in children could be obtained. The general adverse reactions found in adults also apply to children: gastrointestinal, cutaneous/allergic, pulmonary, hepatic, haematological and neurological reactions. Karpman and Kurzrock quoted incidences and incidence rates for gastrointestinal and allergic side effects, but it is not mentioned how these were calculated. The incidence rate of nausea and vomiting was reported to be 4.4 cases per 100 person-years in children younger than 16 years (Uhari et al., 1996). An incidence of 3.2 % was mentioned for allergic reactions in children younger than 15 years. With respect to pulmonary reactions, the authors referred to a report from the national monitoring centre for adverse drug reactions in Sweden (Holmberg and Boman,

1981). In a 10-year period, 447 cases of pulmonary reactions were reported and only three cases (0.7 %) involved children. All were classified as mild acute reactions which subsided after the therapy was stopped. An epidemiological study in Finland evaluated 921 children who received long-term nitrofurantoin prophylaxis and found no adverse pulmonary reactions over a 10-year period (Uhari et al., 1996). Incidences of hepatotoxicity were not given, but, although hepatic reactions are rare in children, they can be fatal. Nitrofurantoin is contraindicated in the first few months of life because of the inability of the immature liver to handle oxidative stress. This has been illustrated by a fatal incident of haemolytic anaemia in a new-born child after maternal treatment with nitrofurantoin at the end of pregnancy. Peripheral neuropathy is the most common neurological adverse reaction in children and it has also been described in an evaluation of the reporting system of the US Food and Drug Administration for adverse drug reactions (Coraggio et al., 1989).

Rascher and Neubert (2012) studied the efficacy and safety of the prophylaxis of recurrent urinary tract infections in children in Germany, where long-term prophylaxis with nitrofurantoin is allowed to start at the age of 3 months and may be continued for a maximum period of 6 months. The prophylactic dose varies from 1 to 2 mg/kg b.w. per day. The use of nitrofurantoin in Germany from 1982 to 2009 led to 222 registered events. Six of these events (2.7 %) were observed in children aged between 4 and 9 years. A causal connection with nitrofurantoin therapy could be established for two events: lung fibrosis in a 9-year-old girl and hepatitis in an 8-year-old girl. No increase in adverse reactions in children could be observed during this 27-year period.

Stricker et al. (1988) reviewed hepatic injuries associated with the use of nitrofurantoin and nifurtoinol (hydroxymethyl-nitrofurantoin) that had been reported since 1963 to the Netherlands Centre for Monitoring of Adverse Reactions to Drugs. In this period of 25 years, 50 cases were associated with nitrofurantoin and, in 38 cases, a causal relationship was considered to be likely. Criteria for the probability of this causality were given. In all cases, the daily dose ranged from 100 to 400 mg. Clinical, biochemical and histopathological findings were reported. Acute hepatic injury was observed in 25 cases, whereas 13 cases presented a chronic type of reaction. Based on the estimated sales figures in the Netherlands (1977 to 1986), the authors estimated an incidence of symptomatic nitrofurantoin-induced hepatic injury of approximately 0.020 to 0.035 %. The authors state that the mechanism of nitrofurantoin-induced hepatic injury seemed to be immunoallergic. This suggestion was also addressed by Björnsson et al. (2010) who investigated drug-induced autoimmune hepatitis among patients of the Mayo Clinic in the USA. Over a period of 10 years (1997–2007), 1 536 patients were diagnosed with autoimmune hepatitis (AIH) and, after applying new exclusion criteria, a total of 261 well-characterised AIH cases were identified. Out of these 261 patients, 11 cases (4.2 %) were related to the use of nitrofurantoin. Besides various clinical and biochemical findings, 8 out of 11 nitrofurantoin patients had abnormalities on hepatic imaging (liver atrophy), a finding seen in only 8 out of 33 of a random sample of the rest of the AIH group.

Nitrofurazone is still used topically for wounds, burns and skin infections. Glascock et al. (1969) reviewed the literature from 1945 to 1965, inclusive, on the topical use of nitrofurazone. The concentrations in the various formulations varied from 0.02 to 1.0 %. During this 20-year period, more than 1 000 reports were published, but only 136 studies provided both the number of patients treated and the number of those who showed allergic reactions. These studies comprised a total of 15 162 treated patients, of which 176 patients (1.2 %) had allergic reactions to the formulation containing nitrofurazone. No distinction was made for allergic reactions that could be attributed to the constituents of the vehicles (e.g. polyethylene glycols) used in the nitrofurazone formulations. Bajaj and Gupta (1986) studied the incidence of contact hypersensitivity to 15 antibacterial agents applied topically. This study includes data gathered from January 1980 to December 1983 and is composed of 390 patients suspected of contact dermatitis to topical antibacterial agents. Patch tests were carried out with various commercially available drugs. In the case of nitrofurazone, 390 patients were tested with patches containing 0.2 % nitrofurazone (formulation not known). From this group, 93 patients were tested with both ointment and powder containing nitrofurazone (concentration unknown, probably 0.2 %). Out of these 390 patients, 141 patients (36.2 %) showed hypersensitive reactions. Out of the 93 patients tested with two formulations, 30 patients (32.2 %) gave positive reactions to ointment and

32 patients (34.4 %) gave positive reactions to powder, both within 48 hours. Among the 15 bacterial agents tested, nitrofurazone was the most common sensitiser. The authors stated that the role of the vehicle (bases and preservatives) could not be tested, but, in the case of nitrofurazone, the two different formulations did not show a significant difference in sensitisation.

No reviews of **nifursol** were identified because nifursol has not been used in human medicine (see Section 1.3.1).

In conclusion, furazolidone and nitrofurantoin have the following adverse reactions in common: gastrointestinal, neurological, systemic, dermatological and haematological reactions and adverse reactions on vital organs such as the heart, lung, liver and kidney. The most common side effects of furazolidone and nitrofurantoin were gastrointestinal reactions: nausea and/or vomiting and abdominal pain. In contrast to nitrofurantoin, no deaths have been associated with adverse reactions to furazolidone. In the case of nitrofurantoin, severe pulmonary side effects were, on rare occasions, fatal. Both drugs have been associated with haemolytic anaemia, observed in patients deficient in G6PD. Because nitrofurantoin has a long history of therapeutic use, much more information on adverse reactions is available than for the other nitrofurans. The topical use of nitrofurazone may lead to allergic reactions.

8.4.2. Epidemiological data on nitrofurans

Only a limited number of epidemiological studies on nitrofurans have been published and these relate primarily to nitrofurantoin.

Selby et al. (1989) used pharmacy records for a cohort of 143 574 patients in California, USA, for the period 1969–1973 to test the association of 215 drugs with subsequent incidence of cancers at 56 sites in the human body over a period of up to 15 years. In total, 1 305 patients used systemic **nitrofurantoin** and three cases of cancers of the nervous system were recorded; 0.6 cases would be expected for this group. In addition, 317 patients used topical **nitrofurazone** and no associations with cancers were recorded for this group.

In Finland, a study was undertaken to compile data on adverse reactions to long-term antimicrobial therapy for recurrent urinary tract infections in children (Uhari et al., 1996). From 16 409 children (< 16 years of age) who had received long-term antimicrobial therapy during the period 1976–1985, a sample of 1 607 girls and 218 boys were included in the study. In the study group, 5 066 (girls) and 607 (boys) treatments were received and the mean duration of each course of treatment was 316 days. Adverse reactions were recorded in 10.4 % of the courses of treatment and 8.2 % of the courses of treatment were discontinued. Common adverse reactions to nitrofurantoin were nausea and vomiting at a rate of 4.4 (95 % confidence interval 3.4–5.4) per 100 person-years. Children of less than 2 years of age had adverse reactions to nitrofurantoin more often than those receiving treatment with sulphonamides, while the reverse occurred for children aged 2–15 years. Most adverse reactions occurred during the first 6 months of treatment and no serious life-threatening adverse reactions were observed. The conclusion from the study was that nitrofurantoin, and sulphonamides, are safe drugs for long-term preventative therapy.

A prospective study was undertaken in the USA (Chalasani et al., 2008) to investigate, *inter alia*, the causative relationship of prescription medications with drug-induced liver injury. In total, 300 patients (2 years of age or older) were included in the study; 93 % were adults (≥ 18 years), 18 % were older than 65 years and 60 % were female. A single prescription medication was implicated in 217 cases (73 %) and one of the more common implicated agents was nitrofurantoin (n = 13); the other most common implicated agents were amoxicillin/clavulanate (n = 23), isoniazid (n = 13) and trimethoprim/sulphamethoxazole (n = 13). Of the 26 patients who died or had a liver transplant within 6 months following recognition of drug-induced liver injury, nitrofurantoin was the implicated agent in two patients, with a final causality score of ‘very likely’.

In the Netherlands, a study was undertaken to establish if women treated with nitrofurantoin for urinary tract infections and renal impairment were at a higher risk of ineffectiveness of treatment and/or serious adverse effects than women without renal impairment (Geerts et al., 2013). A cohort of 21 317 women treated with nitrofurantoin and a cohort of 7 926 women treated with trimethoprim were included in the study. Ineffectiveness of treatment was defined as use of another antimicrobial within 1 month of the start of treatment with nitrofurantoin and the definition of a serious adverse event was one leading to hospitalisation within 90 days. The incidence density for ineffectiveness of treatment was not higher for nitrofurantoin-treated women (5.4 per 1 000 person-days) than for trimethoprim-treated women (6.3 per 1 000 person-days), and moderate renal impairment (estimated glomerular filtration rate of 30–49 mL/min/1.73 m^2) was not associated with ineffectiveness of treatment. The incidence density for serious adverse events was 0.02 per 1 000 person-days for nitrofurantoin treatment, compared with 0.01 per 1 000 person-days for trimethoprim treatment and, in patients with renal impairment (< 50 mL/min/1.73 m^2), the risk of pulmonary adverse events significantly increased for nitrofurantoin treatment; no such event was reported for trimethoprim-treated patients.

Two studies are reported on the effect of nitrofurantoin treatment during pregnancy for urinary tract infections on the teratogenic risk to the fetus. In the first study, in Hungary, the outcome for three groups of pregnant women was studied (Czeizel et al., 2001). In total, 1 079 (2.8 %) of 38 151 women who had new-born infants without any congenital abnormalities (population control group), 774 (3.4 %) of 22 865 women who had new-born infants or fetuses with congenital abnormalities and 23 (2.8 %) of 812 women who had new-born infants or fetuses with Down's syndrome (patient controls) had been treated with nitrofurantoin. Although the incidence (3.4 %) of nitrofuran treatment was higher for the group of women who had new-born infants or fetuses with congenital abnormalities than for the control groups (2.8 %), when biases are excluded, no increased teratogenic potential was found for nitrofurantoin treatment. This study concluded that treatment with nitrofurantoin during pregnancy does not present a detectable teratogenic risk to the fetus.

In the second study, in Norway, the outcome in terms of (1) an increased rate of malformations following first-trimester exposure to nitrofurantoin and (2) an increased rate of negative pregnancy outcomes following exposure to nitrofurantoin during the last 30 days of pregnancy was studied (Nordeng et al., 2013). The incidence of malformations was 31 (2.3 %) of 1 334 women who had been dispensed nitrofurantoin during the first trimester, compared with 162 (2.8 %) of 5 800 women in the disease control group, i.e. women who had been dispensed pivmecillinam during the first trimester, giving an odds ratio of 0.79 (95 % confidence interval 0.51–1.23). The incidence of neonatal jaundice was 103 (10.8 %) of 959 women who had been dispensed nitrofurantoin during the last 30 days of pregnancy, compared with 10 336 (8.1 %) of 127 507 unexposed women, giving an odds ratio of 1.31 (95 % confidence interval 1.02–1.70). This study concluded that the teratogenic potential of nitrofurantoin is low, but use in late pregnancy may increase the risk of neonatal jaundice.

In conclusion, positive associations of nitrofurantoin, given at therapeutic doses, were reported in individual studies with adult cancers of the nervous system, with drug-induced liver injury and with increased risk of pulmonary adverse events in patients with renal impairment. A study of long-term therapy with nitrofurantoin for recurrent urinary tract infections in children found no association with life-threatening adverse reactions. Two studies reported that treatment of women with nitrofurantoin during pregnancy does not give rise to an increased teratogenic risk for the fetus, although one study found that treatment with nitrofurantoin during the last 30 days of pregnancy may increase the risk of neonatal jaundice.

8.5. Considerations of critical effects, dose–response modelling and possibilities for derivation of a health-based guidance value

Nitrofurans have a short half-life due to extensive metabolism and, therefore, they do not occur generally as residues in foods of animal origin. Reactive metabolites are formed that are able to bind covalently to tissue macromolecules and, when such animal tissues are consumed as food, the side-

chains may be released from these metabolites under the acidic conditions of the human stomach, namely AOZ, AMOZ, AHD, SEM and DNSH in the case of furazolidone, furaltadone, nitrofurantoin, nitrofurazone and nifursol, respectively. Owing to the long half-lives of bound metabolites, these releasable side-chains are also used as marker metabolites. At least in the case of furazolidone, it was shown that AOZ can also be released in the stomach of pigs treated with furazolidone, and that AOZ itself is able to form protein-bound adducts in pig tissues which can be hydrolysed to give free AOZ again in the stomach of the consumer. This was shown in studies with isolated pig hepatocytes incubated with AOZ. This metabolic pathway also offers an explanation for the fact that AOZ could be released from tissues of rats that were fed with protein-bound residues of furazolidone (see Section 8.1.5).

As only few, if any, toxicity studies were available for the nitrofuran marker metabolites, the CONTAM Panel, in addition to the evaluation of the toxicological information for the five nitrofuran marker metabolites, also evaluated the toxicological information for the parent compounds. For all five nitrofurans considered in this opinion, studies on their carcinogenic effects were available. A description of the relevant benign and malignant tumours can be found in Section 8.2.7. For the hazard characterisation of the carcinogenic effects, the CONTAM Panel focused on the evaluation of the malignant tumours only.

8.5.1. Furazolidone and AOZ

Several studies reported that **furazolidone** is carcinogenic in experimental animals (see Section 8.2.7). Furazolidone induced bronchial adenocarcinomas in male and female mice, malignant mammary tumours in female rats, neural astrocytomas in male rats and sebaceous adenocarcinomas in male and female rats. There is no information on the carcinogenicity in humans. Furazolidone induced mutations in bacterial test systems and caused SCE and DNA strand breaks in mammalian cells *in vitro*. *In vivo*, micronucleus studies gave negative or equivocal results, but an increase in SCE frequency was observed. Based on these data, the CONTAM Panel concluded that furazolidone is genotoxic *in vitro* and may possibly be genotoxic *in vivo*, and considered furazolidone to be a substance which is genotoxic and carcinogenic. Therefore, the CONTAM Panel concluded that the derivation of a health-based guidance value (HBGV) is not appropriate, and decided to apply an MOE approach for its risk characterisation.

The CONTAM Panel considered the tumour data from four carcinogenicity studies to be suitable for dose–response modelling:

1. Halliday et al. (1974)—bronchial adenocarcinomas observed in male and female Swiss MBR/ICR mice;
2. King et al. (1972a) and Halliday et al. (1973a)—malignant mammary tumours (adenocarcinomas and carcinosarcomas) observed in female Sprague–Dawley rats;
3. King et al. (1972b) and Halliday et al. (1973b)—mammary adenocarcinomas observed in female Fischer 344 rats;
4. King et al. (1972b) and Halliday et al. (1973b)—malignant mammary tumours (adenocarcinomas and carcinosarcomas) in female and neural astrocytomas observed in male Sprague–Dawley rats.

The study by Siedler and Searfoss (1966) described in Section 8.2.7 was not suitable for dose–response modelling, as only one dose was included.

Table I.9 (Appendix I Section I.1) shows the benchmark dose (BMD) results for the four considered tumour types (bronchial adenocarcinomas, malignant mammary tumours, mammary adenocarcinomas and neural astrocytomas) and details are shown in Appendix I, Section I.1. The CONTAM Panel noted that, in all four carcinogenicity studies, there was considerable mortality before the end of the studies. When animals died before the end of the study without having developed tumours, it remains

unknown if they would have developed tumours had they not died. This creates an additional uncertainty for the dose–response relationship which cannot be accounted for in the statistical analysis (with the information available). Therefore, the benchmark dose lower (BMDL) and upper (BMDU) confidence limits should be considered as indicative. From the results, the CONTAM Panel selected the lowest $BMDL_{10}$ value (lower 95 % confidence limit or a benchmark response of 10 % extra risk) of 3.5 mg/kg b.w. per day as a reference point for the carcinogenic effects of furazolidone.

For **AOZ**, the marker metabolite of furazolidone, there is no information on its carcinogenicity, but the limited data indicate that it is genotoxic *in vitro* and possibly *in vivo*. Because of its genotoxicity, the CONTAM Panel concluded that the derivation of an HBGV for AOZ is not appropriate. The CONTAM Panel assumed that the carcinogenicity of furazolidone could be caused by AOZ and, therefore, the $BMDL_{10}$ value of 3.5 mg/kg b.w. per day for furazolidone can be used for AOZ.

Because the residues of furazolidone are expressed as its marker metabolite AOZ, and the molecular weights of furazolidone and AOZ differ, the CONTAM Panel concluded that the reference point to be used in the risk characterisation of the carcinogenic effects of residues of furazolidone, expressed as AOZ, is 102 / 225 × 3.5 = 1.6 mg/kg b.w. per day.

For non-neoplastic effects of furazolidone, only limited information is available from long-term/carcinogenicity studies, such as effects on red blood cell parameters and increases in adrenal cortex and thyroid atrophy. For the most sensitive effect—a reduction in the number of red blood cells, observed at the end of the chronic study in Sprague–Dawley rats (Halliday et al., 1973a)—the CONTAM Panel performed BMD analysis because this approach is a scientifically more advanced method to the NOAEL approach for deriving a reference point, as it makes extended use of available dose–response data and it quantifies the uncertainties in the dose–response data, resulting, overall, in a more consistent reference point (EFSA, 2009). For the reduction in the number of red blood cells, a $BMDL_{05}$ of 0.1 mg/kg b.w. per day has been derived (see Appendix I, Section I.2). This value can be applied as reference point for the non-neoplastic effects of furazolidone.

There is only limited information on the toxicity of AOZ (see Section 8.2.2). In two 90-day studies in rats and dogs, effects on red blood cell parameters and the spleen were found for rats at a dose of 6 mg/kg b.w. per day and for dogs at the lowest tested dose of 1 mg/kg b.w. per day (NOTOX, 1995b; Brinck et al., 1995). In addition, in male and female dogs, dose-related effects on enzymes in blood (ALP, AST) and bilirubin were found (Brinck et al., 1995). BMD analysis was performed on the effects on the red blood cell count and serum levels of ALP, AST and bilirubin in dogs (see Appendix I, Section I.3). For the effect on red blood cells in dogs, a $BMDL_{05}$ of 0.04 mg/kg b.w. per day was derived. For the other effects, the lowest $BMDL_{05}$ was 0.02 mg/kg b.w. per day for the effect of AOZ on ALP.

Because the residues of furazolidone are expressed as its marker metabolite AOZ, the CONTAM Panel concluded that the $BMDL_{05}$ of 0.02 mg/kg b.w. per day for AOZ can be used as a reference point in the risk characterisation of the non-neoplastic effects of residues of furazolidone, expressed as AOZ.

8.5.2. Furaltadone and AMOZ

For **furaltadone**, there are two limited studies (using only one dose level) showing that it induces malignant mammary tumours in rats (see Section 8.2.7). With regard to its genotoxicity, it is a strong bacterial mutagen and it induces mutations in mammalian cells *in vitro* (see Section 8.2.6). Although the information is limited, the CONTAM Panel concluded that furaltadone is considered to be a genotoxic carcinogen, for which the derivation of an HBGV is inappropriate, and therefore decided to apply an MOE approach for its risk characterisation.

Two chronic studies were available on the carcinogenicity of furaltadone, and both used only one dose level. In the study of Cohen et al. (1973), female Sprague–Dawley rats received an oral dose of 54 mg/kg b.w. per day in the diet. In this study, a rather high incidence of malignant mammary

tumours (25 out of 32) was observed. In the other study, in which a single dose of 85 mg/kg b.w. per day was administered to female Holtzman rats in the diet, the incidence of malignant mammary tumours was much lower: 3 out of 35. To be prudent, the CONTAM Panel used the Cohen study to estimate a $BMDL_{10}$. To circumvent the problem of the single dose in the Cohen data, a dose–response analysis for the tumour data was performed, assuming that the shape parameter of the fitted model was the same as for furazolidone. This resulted in a rather wide BMD confidence interval of 0.03 to 40 mg/kg b.w. per day (see Appendix I, Section I.4), indicating a large uncertainty in the BMD estimate. Given the large difference between the BMDL and the BMDU, the CONTAM Panel considered that the available data do not provide a suitable basis for deriving a reference point for the carcinogenic effects of furaltadone.

Comparing the confidence interval for furaltadone with that for furazolidone (25–86 mg/kg b.w. per day), the CONTAM Panel noted that, although they overlap to some extent, the interval for furaltadone reflects a lower dose range. This indicates that furaltadone may be more potent than furazolidone. This, however, contradicts the study by Siedler and Searfoss (1966) on Holtzman rats in which both compounds were studied. A slightly higher but equimolar dose of furaltadone, i.e. 85 mg/kg b.w. per day (0.26 mmol/kg b.w. per day), compared with 57 mg/kg b.w. per day (0.25 mmol/kg b.w. per day) of furazolidone induced a similar, low incidence of mammary adenocarcinomas (furaltadone, 3 out of 35; furazolidone, 5 out of 35). Recognising the limitations of the available data, the CONTAM Panel concluded that there are no clear indications that furaltadone is more potent than furazolidone with respect to the induction of mammary adenocarcinomas.

For **AMOZ**, the marker metabolite of furaltadone, no information on carcinogenicity was identified, but the limited data that are available on its mutagenicity/genotoxicity indicate that it is non-genotoxic *in vitro*. The CONTAM Panel could not conclude on the carcinogenicity of AMOZ.

There is no information on non-neoplastic effects of furaltadone or AMOZ that could be used for the derivation of a reference point for the risk characterisation.

8.5.3. Nitrofurantoin and AHD

In several long-term studies in mice and rats (see Section 8.2.7), **nitrofurantoin** induced predominantly benign tumours (e.g. ovarian tubular adenomas, mammary fibroadenomas). In one study, a few malignant tumours were observed in male rats (renal tubular carcinomas in two high-dose males, and osteosarcomas in one low-dose male and two high-dose males). Based on these observations, the CONTAM Panel concluded that the evidence that nitrofurantoin is carcinogenic in experimental animals is limited. *In vitro*, nitrofurantoin induced mutations, DNA damage and chromosomal aberrations. *In vivo*, it has been shown to induce DNA damage in multiple organs, micronuclei formation in mice and gene mutations in a transgenic mouse mutation assay. In humans (children), there are indications that long-term prophylactic treatment might induce SCEs in lymphocytes. The CONTAM Panel concluded that nitrofurantoin is genotoxic *in vivo.* Although there is only limited evidence for the carcinogenicity of nitrofurantoin, the CONTAM Panel concluded that, to be prudent, the compound should be considered a substance which is genotoxic and carcinogenic, for which the derivation of an HBGV is not appropriate. It therefore decided to apply an MOE approach for risk characterisation of nitrofurantoin.

Based on the low incidence of osteosarcomas observed in male rats (NTP, 1989), the CONTAM Panel derived a $BMDL_{10}$ of 61 mg/kg b.w. per day as a reference point for the carcinogenic effect of nitrofurantoin (see Appendix I, Section I.5). The CONTAM Panel recognised that this can be considered a conservative approach because, owing to the very low incidence of the osteosarcomas, the $BMDU_{10}$ is infinite.

For **AHD**, the marker metabolite of nitrofurantoin, there is no information on carcinogenicity and limited information on genotoxicity. The CONTAM Panel considered that AHD may play a role in the

carcinogenicity of nitrofurantoin and therefore the $BMDL_{10}$ value of 61 mg/kg b.w. per day for nitrofurantoin can be used for the risk characterisation of the carcinogenic effects of AHD.

Because the residues of nitrofurantoin are expressed as its marker metabolite AHD, and the molecular weights of nitrofurantoin and AHD differ, the CONTAM Panel concluded that the reference point to be used in the risk characterisation of the carcinogenic risks of residues of nitrofurantoin, expressed as AHD, is 115/238 × 61 = 29.5 mg/kg b.w. per day.

Regarding the non-neoplastic effects of nitrofurantoin, the testes and in particular spermatogenesis were considered to be the most sensitive targets, with large effects (up to two-fold) seen at the lowest tested oral dose of 10 mg/kg b.w. per day (see Section 8.2.4.1). A BMD analyses was performed for the effect of nitrofurantoin on spermatogenic index, number of tubuli containing spermatozoa, time of motility of spermatozoa and concentration of spermatozoa as reported by Yunda et al. (1974). However, as there were only three dose groups (including controls), the estimated BMD confidence intervals were unstable, i.e. depended on the chosen start values of the parameters, and the CONTAM Panel concluded that (with current statistical methodology) no reliable BMD confidence intervals could be derived. Instead, the CONTAM Panel selected the lowest dose tested of 10 mg/kg b.w. per day at which effects on spermatogenesis were observed as a reference point for the non-neoplastic effects of nitrofurantoin, noting that the effects at this dose are substantial.

For AHD, the marker metabolite of nitrofurantoin, there is no information on non-neoplastic effects. The CONTAM Panel assumes that the non-neoplastic effects of nitrofurantoin may be caused by AHD and therefore the lowest dose tested of 10 mg/kg b.w. per day at which effects on spermatogenesis were observed for nitrofurantoin can be used for AHD.

Because the residues of nitrofurantoin are expressed as its marker metabolite AHD, and the molecular weights of nitrofurantoin and AHD differ, the CONTAM Panel concluded that the reference point to be used in the risk characterisation of the non-neoplastic effects of residues of nitrofurantoin, expressed as AHD, is 115 / 238 × 10 = 4.8 mg/kg b.w. per day.

8.5.4. Nitrofurazone and SEM

Following long-term oral administration to mice and rats, **nitrofurazone** increased the incidence of benign tumours such as granulosa cell adenomas and benign mixed tumours of the ovary in mice and mammary fibroadenomas in rats. In one study with rats, a non-dose-related increase in carcinomas of the preputial gland was observed, but the combined incidence of preputial gland adenomas and carcinomas, which was considered to be the most appropriate parameter for this type of tumour, was not affected (see Section 8.2.7). Based on this observation, the CONTAM Panel concluded that there is no evidence of the carcinogenicity of nitrofurazone in mice, and that evidence for the carcinogenicity of nitrofurazone in rats is equivocal. Nitrofurazone was genotoxic *in vitro* but no conclusion on the *in vivo* genotoxicity can be drawn. Therefore, no clear conclusion on the genotoxicity and carcinogenicity of nitrofurazone can be drawn. In addition, the available information is not suitable to derive a reliable reference point for the possible carcinogenicity of nitrofurazone in rats.

SEM, the marker metabolite of nitrofurazone, increased the incidence of lung tumours in two limited studies in mice, using only one dose level. In one study, the lung tumours were not specified; in the other study, an increase in malignant lung tumours in female mice was indicated in only a semi-quantitative way. In the two available rat studies, no increase in tumour incidence was found (see Section 8.2.7). Recognising the shortcomings of most of these studies, the CONTAM Panel concluded that there is limited evidence that SEM is carcinogenic in mice, and that there is no evidence in rats. The Panel noted that this is contrary to the response of nitrofurazone. SEM is mutagenic in bacteria and in mammalian cells *in vitro*, and showed clastogenic potential *in vivo*, but without a dose–response relationship. The CONTAM Panel concluded that SEM is genotoxic *in vitro*, but that no

conclusion on its genotoxicity *in vivo* can be drawn. The available information is too limited to conclude on a reference point for carcinogenicity of SEM in mice.

Because of the limitations in the database, the CONTAM Panel cannot derive a reference point that can be used in the risk assessment of the carcinogenic effects of residues of nitrofurazone, expressed as its marker metabolite SEM.

In repeated-dose toxicity studies (see Section 8.2.2) with nitrofurazone, the main toxic effects were on reproductive organs. Based on effects on the testes in rats, a NOAEL of 13.5 mg/kg b.w. per day was identified in a 13-week study. In studies on spermatogenesis (see Section 8.2.4.1), effects on the testes and the epididymis were seen, but the most sensitive endpoint was massive spermiation failure observed in rats at the lowest tested dose of 12.5 mg/kg b.w. per day administered orally for up to 12 weeks. In addition, reproductive toxicity studies (see Section 8.2.4.3) in mice confirmed that the testis is the target organ, as disruption of fertility (related to degeneration of the seminiferous tubules), abnormal sperm morphology and a reduced testicular spermatid concentration were seen at the lowest tested dose of 14 mg/kg b.w. per day. Testes degeneration was also observed in chronic toxicity studies (see Section 8.2.7) in nearly all dosed rats (49 out of 50 and 47 out of 50 for doses of 11 and 24 mg/kg b.w. per day, respectively, versus 12 out of 50 in the controls). For a number of endpoints (testis and epididymis weight and testicular and epididymal sperm number), BMD analysis was performed, and the lowest $BMDL_{05}$ value of 4.6 mg/kg b.w. per day was obtained for the decrease in epididymis weight in rats (see Appendix I, Section I.6). The CONTAM Panel noted that this $BMDL_{05}$ value is not much lower than dose levels at which strong effects were seen in rats, i.e. massive spermiation failure at 12.5 mg/kg b.w. per day and testis degeneration at 11 mg/kg b.w. per day, but the data for both of these endpoints were not suitable for a BMD analysis.

In a 90-day study in which rats were orally administered SEM, a number of severe effects such as deformation of limbs and osteochondral lesions were observed in all dose groups, including the lowest dose of 23 mg/kg b.w. per day (see Section 8.2.2). In a chronic study with rats, disarrangement of chondrocytes in bones and degeneration of the articular cartilage in knee joints were observed, with a NOAEL of 0.6 mg/kg b.w. per day (see Section 8.2.7). In a teratogenicity study, an increase in cleft palate was seen at the lowest tested dose of 10 mg/kg b.w. (see Section 8.2.4.2). Upon BMD analysis (see Appendix I, Section I.7), a lowest $BMDL_{10}$ for effects on bones was derived of 1.0 mg/kg b.w. per day.

The CONTAM Panel noted that, in contrast to nitrofurazone, the available data for SEM do not indicate an effect on the testes, but concluded that the $BMDL_{10}$ of 1.0 mg/kg b.w. per day for SEM could be used as a reference point for the risk characterisation of the non-neoplastic effects of residues of nitrofurazone, expressed as its marker metabolite SEM.

8.5.5. Nifursol and DNSH

Based on the limited available information on **nifursol**, the CONTAM Panel concluded that there is no clear indication that the compound is carcinogenic. *In vitro*, nifursol is mutagenic in bacteria and induces chromosomal aberrations, but *in vivo* clastogenicity studies and an *in vivo* mutation assay with transgenic mice gave clear negative results. Based on these data, the CONTAM Panel concludes that nifursol is genotoxic *in vitro*, but not *in vivo*. However, the available toxicological information is too limited to derive an HBGV and the CONTAM Panel decided to apply an MOE approach for the risk characterisation of nifursol. For several endpoints, i.e. effects on red blood cell parameters and on spleen weight observed in a 13-week rat study (see Section 8.2.2), and effects on liver weight observed in a chronic study with rats (see Section 8.2.7), a BMD analysis was carried out (see Appendix I, Section I.8). A lowest $BMDL_{05}$ of 11 mg/kg b.w. per day was derived for effects on liver weight. The CONTAM Panel concluded that this value could be used as a reference point for the non-neoplastic effect of nifursol.

For **DNSH**, the marker metabolite of nifursol, there is no information on its toxicity, carcinogenicity or mutagenicity/genotoxicity. Because the residues of nifursol are expressed as its marker metabolite DNSH, and the molecular weights of nifursol and DNSH differ, the CONTAM Panel concluded that the reference point to be used in the risk characterisation of residues of nifursol, expressed as DNSH, is 242/365 × 11 = 7.3 mg/kg b.w. per day.

9. Risk characterisation

The CONTAM Panel considered the nitrofurans furazolidone, furaltadone, nitrofurantoin, nitrofurazone and nifursol in this scientific opinion. Nitrofurans have short half-lives and, therefore, they do not occur generally as residues in foods of animal origin. Reactive metabolites are formed that are able to bind covalently to tissue macromolecules and, when such animal tissues are consumed as food, the side-chains may be released, namely AOZ, AMOZ, AHD, SEM and DNSH. Owing to the long half-lives of bound metabolites, these releasable side-chains are also used as marker metabolites. At least in the case of furazolidone, it was shown that AOZ can also be released in the stomach of pigs treated with furazolidone, and that AOZ itself is able to form protein-bound adducts in pig tissues which can be hydrolysed to give free AOZ again in the stomach of the consumer (see Section 8.1.5).

The CONTAM Panel considered the application of a read-across approach between the nitrofuran marker metabolites, but because of the different critical effects observed, the CONTAM Panel characterised the risk for each of the marker metabolites separately. As nitrofuran marker metabolites are hydrazines, which are excluded from the threshold of toxicological concern approach, such an approach was not applied for the risk characterisation.

Only limited occurrence data on nitrofurans and their marker metabolites in food were available for this opinion (see Section 5.2). The CONTAM Panel concluded that these data are too limited to carry out a reliable human dietary exposure assessment. Therefore, the CONTAM Panel cannot characterise the risk of actual exposure to nitrofuran marker metabolites.

9.1. Evaluation whether a reference point for action of 1 µg/kg for nitrofuran metabolites as defined in the legislation in food of animal origin is adequate to protect public health

To evaluate whether or not an RPA of 1 µg/kg for nitrofuran metabolites, as defined in the legislation (Commission Decision 2002/657/EC and Commission Decision 2005/34/EC), in foods of animal origin is adequate to protect public health, the CONTAM Panel considered the exposure to nitrofuran marker metabolites resulting from illicit nitrofuran use. Such exposure is covered by exposure scenario 1A, in which foods of animal origin (excluding milk and dairy products) are contaminated with one nitrofuran marker metabolite at a concentration equal to the RPA level of 1 µg/kg. These are mainly meat and meat products, fish and fish products, eggs and egg products and honey.

Based on scenario 1A, the median chronic dietary exposure for AOZ, AMOZ, AHD, SEM or DNSH across dietary surveys for the average consumer would be 5.5 and 2.6 ng/kg b.w. per day for toddlers (the highest exposed population group) and adults, respectively. The minimum and maximum chronic dietary exposures across dietary surveys for the average consumer would be 3.3 and 8.0 ng/kg b.w. per day, respectively, for toddlers and 1.9 and 4.3 ng/kg b.w. per day, respectively, for adults (see Table 5).

When comparing the median chronic dietary exposure to the furazolidone marker metabolite **AOZ**, based on scenario 1A, across dietary surveys for the average consumer with the $BMDL_{10}$ for carcinogenicity of furazolidone, expressed as AOZ (1.6 mg AOZ/kg b.w. per day), the MOE would be about 2.9×10^5 for toddlers and 6.2×10^5 for adults. For the minimum and maximum chronic dietary exposures across dietary surveys for the average consumer, the MOEs for toddlers would be about 4.8×10^5 and 2.0×10^5, respectively, and for adults would be about 8.4×10^5 and 3.7×10^5, respectively (Table 9).

For substances that are both genotoxic and carcinogenic, the EFSA Scientific Committee proposed that an MOE of 10 000 or higher, if based on the $BMDL_{10}$ from an animal carcinogenicity study, would be of low concern from a public health point of view (EFSA, 2005). Considering that the calculated MOEs for carcinogenicity would be of the order of 10^5, they are of low concern. Furthermore, they are considered sufficiently large to cover the additional uncertainty regarding the carcinogenicity data and the $BMDL_{10}$, and the uncertainty related to the assumption that the carcinogenicity of furazolidone is caused by its metabolite AOZ.

Table 9: Mean chronic dietary exposure to nitrofuran marker metabolites under scenario 1A and the calculated margins of exposure for toddlers and adults

Mean chronic dietary exposure to nitrofuran marker metabolites (ng/kg b.w. per day) under scenario 1A[a]			Toddlers		Adults	
			Median	Range	Median	Range
			5.5	3.3–8.0	2.6	1.9–4.3
Substance	**Reference point**		**MOE for toddlers**		**MOE for adults**	
	Description	**mg/kg b.w. per day**	**Median**	**Range**	**Median**	**Range**
AOZ	$BMDL_{10}$ (neoplastic)—bronchial adenocarcinomas, mice[b]	1.6	2.9×10^5	$2.0–4.8 \times 10^5$	6.2×10^5	$3.7–8.4 \times 10^5$
	$BMDL_{05}$ (non-neoplastic)—effect on ALP, dogs	0.02	3.6×10^3	$2.5–6.1 \times 10^3$	7.7×10^3	$4.7–11 \times 10^3$
AMOZ	BMDL (neoplastic)	Not identified	–	–	–	–
	BMDL (non-neoplastic)	Not identified	–	–	–	–
AHD	$BMDL_{10}$ (neoplastic)—osteosarcomas, male rats[c]	29.5	5.4×10^6	$3.7–8.9 \times 10^6$	1.1×10^7	$0.7–1.6 \times 10^7$
	Effect dose (non-neoplastic)—spermatogenesis, rats[d]	4.8	8.7×10^5	$6.0–15 \times 10^5$	1.8×10^6	$1.1–2.5 \times 10^6$
SEM	BMDL (neoplastic)	Not identified	–	–	–	–
	$BMDL_{10}$ (non-neoplastic)—effects on bones, rats	1.0	1.8×10^5	$1.3–3.0 \times 10^5$	3.8×10^5	$2.3–5.3 \times 10^5$
DNSH	BMDL (neoplastic)	Not applicable	–	–	–	–
	$BMDL_{05}$ (non-neoplastic)—effect on liver weight[e]	7.3	1.3×10^6	$0.9–2.2 \times 10^6$	2.8×10^6	$1.7–3.8 \times 10^6$

AHD: 1-aminohydantoin; AMOZ: 3-amino-5-methylmorpholino-2-oxazolidinone; AOZ: 3-amino-2-oxazolidinone; BMDL: benchmark dose lower confidence limit; b.w.: body weight; DNSH: 3,5-dinitrosalicylic acid hydrazide; MOE: margin of exposure; RPA: reference point for action; SEM: semicarbazide.

(a): Scenario 1A contains foods of animal origin, excluding milk and dairy products, that are contaminated with one nitrofuran marker metabolite at a concentration equal to the RPA value of 1 µg/kg (meat and meat products, fish and fish products, eggs and egg products and honey).

(b): $BMDL_{10}$ calculated from data on furazolidone; value should be considered as indicative.

(c): $BMDL_{10}$ calculated from data on nitrofurantoin.

(d): Effect dose identified from study on nitrofurantoin.

(e): $BMDL_{05}$ calculated from data on nifursol.

For non-neoplastic effects, the CONTAM Panel identified a $BMDL_{05}$ of 0.02 mg/kg b.w. per day for the effect on ALP caused by AOZ. When comparing this $BMDL_{05}$ with the median chronic dietary exposure to AOZ, based on scenario 1A, across dietary surveys for the average consumer, the MOE would be about 3.6×10^3 for toddlers and 7.7×10^3 for adults. For the minimum and maximum chronic dietary exposures across dietary surveys for the average consumer, the MOEs for toddlers would be about 6.1×10^3 and 2.5×10^3, respectively, and for adults would be about 1.1×10^4 and 4.7×10^3, respectively (Table 9).

The CONTAM Panel noted that MOEs of 100 are often considered of low concern for threshold effects (FAO/WHO, 2009). Considering that the calculated MOEs for the effect on ALP would be of the order of 10^3 or higher, they are considered sufficiently large and do not indicate a health concern for non-neoplastic effects.

The CONTAM Panel concluded that it is unlikely that exposure to food contaminated with AOZ at or below 1 μg/kg represents a health concern.

The CONTAM Panel could not conclude on the carcinogenicity of the furaltadone marker metabolite **AMOZ**. Given that there are no clear indications that furaltadone is more potent than furazolidone with respect to the induction of mammary adenocarcinomas, the CONTAM Panel concluded that the cancer risk from AMOZ, if any, would not be greater than that from AOZ and hence does not indicate a health concern.

The CONTAM Panel could not identify a reference point for non-neoplastic effects for AMOZ and therefore the risk could not be assessed.

When comparing the median chronic dietary exposure to the nitrofurantoin marker metabolite **AHD**, based on scenario 1A, across dietary surveys for the average consumer with the $BMDL_{10}$ for carcinogenicity of nitrofurantoin, expressed as AHD (29.5 mg AHD/kg b.w. per day), the MOE would be about 5.4×10^6 for toddlers and 1.1×10^7 for adults. For the minimum and maximum chronic dietary exposures across dietary surveys for the average consumer, the MOEs for toddlers would be about 8.9×10^6 and 3.7×10^6, respectively, and for adults would be about 1.6×10^7 and 6.9×10^6, respectively (Table 9).

Considering that the calculated MOEs for carcinogenicity would be of the order of 10^6 and higher, they are considered sufficiently large to cover the uncertainty related to the assumption that the carcinogenicity of nitrofurantoin is caused by its metabolite AHD.

When comparing the median chronic dietary exposure to the nitrofurantoin marker metabolite AHD, based on scenario 1A, across dietary surveys for the average consumer with the effect dose[38] on spermatogenesis of nitrofurantoin, expressed as AHD (4.8 mg AHD/kg b.w. per day), the MOE would be about 8.7×10^5 for toddlers and 1.8×10^6 for adults. For the minimum and maximum chronic dietary exposures across dietary surveys for the average consumer, the MOEs for toddlers would be about 1.5×10^6 and 6.0×10^5, respectively, and for adults would be about 2.5×10^6 and 1.1×10^6, respectively (Table 9).

The calculated MOEs for effects on spermatogenesis of AHD are not based on a NOAEL or a BMDL but on an effect level at which the effects are substantial. However, as the MOEs are of the order of 10^5 or higher, they are considered to be sufficiently large and do not indicate a health concern for non-neoplastic effects of AHD.

The CONTAM Panel concluded that it is unlikely that exposure to food contaminated with AHD at or below 1 μg/kg represents a health concern.

[38] Lowest dose tested at which effects on spermatogenesis were observed.

SEM is carcinogenic in mice, but not in rats. However, the available information is too limited to conclude on a reference point for carcinogenicity in mice and the cancer risk cannot be assessed.

For non-neoplastic effects, the CONTAM Panel identified a $BMDL_{10}$ of 1.0 mg/kg b.w. per day for the effect on bones caused by SEM. When comparing this $BMDL_{10}$ with the median chronic dietary exposure to SEM, based on scenario 1A, across dietary surveys for the average consumer, the MOE would be about 1.8×10^5 for toddlers and 3.8×10^5 for adults. For the minimum and maximum chronic dietary exposures across dietary surveys for the average consumer, the MOEs for toddlers would be about 3.0×10^5 and 1.3×10^5, respectively, and for adults would be about 5.3×10^5 and 2.3×10^5, respectively (Table 9).

Considering that the calculated MOEs for the effect of SEM on bones would be of the order of 10^5, they are considered sufficiently large and do not indicate a health concern for non-neoplastic effects.

No information regarding the carcinogenicity is available for the nifursol marker metabolite **DNSH**. Based on the limited available information on nifursol, the CONTAM Panel concluded that there is no clear indication that nifursol is carcinogenic.

For non-neoplastic effects, the CONTAM Panel identified a $BMDL_{05}$ for the effects of nifursol on liver weight, expressed as DNSH (7.3 mg DNSH/kg b.w. per day). When comparing this $BMDL_{05}$ with the median chronic dietary exposure to DNSH, based on scenario 1A, across dietary surveys for the average consumer, the MOE would be about 1.3×10^6 for toddlers and 2.8×10^6 for adults. For the minimum and maximum chronic dietary exposures across dietary surveys for the average consumer, the MOEs for toddlers would be about 2.2×10^6 and 9.1×10^5, respectively, and for adults would be about 3.8×10^6 and 1.7×10^6, respectively (Table 9).

Considering that the calculated MOEs for the effect of DNSH on liver weight would be of the order of 10^5 or higher, they are considered sufficiently large and do not indicate a health concern for non-neoplastic effects.

Overall, the CONTAM Panel concludes that the presence of AOZ, AHD and DNSH in food at or below a level of 1 µg/kg is unlikely to be a health concern. Owing to the lack of appropriate data, the CONTAM Panel cannot assess the cancer risk or the risk of non-neoplastic effects of AMOZ. The presence of SEM in food at or below a level of 1 µg/kg is unlikely to be a health concern for non-neoplastic effects but, owing to the lack of appropriate data, the cancer risk of SEM cannot be assessed.

9.2. Assessment of the appropriateness of applying the reference point for action that is considered adequate to protect public health to other commodities than food of animal origin

No occurrence of AOZ, AMOZ, AHD or DNSH has been reported in foods of non-animal origin. Therefore, the CONTAM Panel considered that this term of reference does not apply to these substances.

Only SEM is reported to occur in foods of non-animal origin owing to its occurrence in the food additive carrageenan, which is used in a large variety of foods of both non-animal and animal origin. Because the available information on SEM is too limited to conclude on a reference point for carcinogenicity, the risk characterisation focuses on non-neoplastic effects only.

To address this term of reference, the CONTAM Panel decided to use exposure scenario 2A, in which foods of non-animal origin for which carrageenan is authorised as an additive are contaminated with SEM at a concentration in the final food product equal to the RPA level of 1 µg/kg.

Based on scenario 2A, the median chronic dietary exposure for SEM across dietary surveys for the average consumer would be 14 and 5.7 ng/kg b.w. per day for toddlers (the highest exposed

population group) and adults, respectively. The minimum and maximum chronic dietary exposures across dietary surveys for the average consumer would be 4.6 and 41 ng/kg b.w. per day, respectively, for toddlers and 3.3 and 13 ng/kg b.w. per day, respectively, for adults (see Table 7).

As the exposure to SEM from dairy products in which carrageenan is used is not covered by scenario 1A or scenario 2A, the CONTAM Panel considered scenario 2C, in which foods of animal origin (including only those milk and dairy products for which carrageenan is authorised as an additive) and foods of non-animal origin (for which carrageenan is authorised as an additive) are contaminated with SEM at a concentration equal to the RPA level of 1 µg/kg.

Based on scenario 2C, the median chronic dietary exposure for SEM across dietary surveys for the average consumer would be 29 and 9.6 ng/kg b.w. per day for toddlers (the highest exposed population group) and adults, respectively. The minimum and maximum chronic dietary exposures across dietary surveys for the average consumer would be 17 and 55 ng/kg b.w. per day, respectively, for toddlers and 6.4 and 16 ng/kg b.w. per day, respectively, for adults (see Table 7).

For non-neoplastic effects, the CONTAM Panel identified a $BMDL_{10}$ of 1.0 mg/kg b.w. per day for the effect on bones caused by SEM. When comparing this $BMDL_{10}$ with the median chronic dietary exposure to SEM, based on scenario 2A, across dietary surveys for the average consumer, the MOE would be about 7.1×10^4 for toddlers and 1.8×10^5 for adults. For the minimum and maximum chronic dietary exposures across dietary surveys for the average consumer, the MOEs for toddlers would be about 2.2×10^5 and 2.4×10^4, respectively, and for adults would be about 3.0×10^5 and 7.4×10^4, respectively (Table 10).

When comparing the $BMDL_{10}$ of 1.0 mg/kg b.w. per day with the median chronic dietary exposure to SEM, based on scenario 2C, across dietary surveys for the average consumer, the MOE would be about 3.4×10^4 for toddlers and 1.0×10^5 for adults. For the minimum and maximum chronic dietary exposures across dietary surveys for the average consumer, the MOEs for toddlers would be about 5.9×10^4 and 1.8×10^4, respectively, and for adults would be about 1.6×10^5 and 6.3×10^4, respectively (Table 11).

The calculated MOEs for the effect of SEM on bones would be of the order of 10^4 or higher. These MOEs are considered to be sufficiently large and, therefore, the CONTAM Panel concludes that the presence of SEM in food at or below a level of 1 µg/kg is unlikely to be a health concern for non-neoplastic effects. Owing to the lack of appropriate data, the CONTAM Panel cannot assess the cancer risk of SEM.

Table 10: Mean chronic dietary exposure to semicarbazide under scenario 2A and the calculated margins of exposure for toddlers and adults

Mean chronic dietary exposure to nitrofuran marker metabolites (ng/kg b.w. per day) under scenario 2A[(a)]			Toddlers		Adults	
			Median	Range	Median	Range
			14	4.6–41	5.7	3.3–13
Substance	**Reference point**		**MOE for toddlers**		**MOE for adults**	
	Description	**mg/kg b.w. per day**	**Median**	**Range**	**Median**	**Range**
SEM	BMDL (neoplastic)	Not identified	–	–	–	–
	$BMDL_{10}$ (non-neoplastic)—effects on bones, rats	1.0	7.1×10^4	$2.4–22 \times 10^4$	1.8×10^5	$0.7–3.0 \times 10^5$

BMDL: benchmark dose lower confidence limit; b.w.: body weight; MOE: margin of exposure; RPA: reference point for action; SEM: semicarbazide.
(a): Scenario 2A contains foods of non-animal origin for which carrageenan is authorised as an additive and contaminated with SEM at a concentration in the final food product equal to the RPA level of 1 µg/kg.

Table 11: Mean chronic dietary exposure to semicarbazide under scenario 2C and the calculated margins of exposure for toddlers and adults

Mean chronic dietary exposure to nitrofuran marker metabolites (ng/kg b.w. per day) under scenario 2C[(a)]			Toddlers		Adults	
			Median	Range	Median	Range
			29	17–55	9.6	6.4–16
Substance	**Reference point**		**MOE for toddlers**		**MOE for adults**	
	Description	**mg/kg b.w. per day**	**Median**	**Range**	**Median**	**Range**
SEM	BMDL (neoplastic)	Not identified	–	–	–	–
	$BMDL_{10}$ (non-neoplastic)—effects on bones, rats	1.0	3.4×10^4	$1.8–5.9 \times 10^4$	1.0×10^5	$0.6–1.6 \times 10^5$

BMDL: benchmark dose lower confidence limit; b.w.: body weight; MOE: margin of exposure; RPA: reference point for action; SEM: semicarbazide.
(a): Scenario 2C contains foods of animal origin—including only those milk and dairy products for which carrageenan is authorised as an additive—and foods of non-animal origin—for which carrageenan is authorised as an additive—which are contaminated with SEM at a concentration equal to the RPA level of 1 µg/kg.

10. Uncertainty analysis

The CONTAM Panel concluded that the available occurrence data on the prohibited substances nitrofurans and their marker metabolites in food were too limited and therefore preclude a reliable human dietary exposure assessment and consequently a detailed evaluation of the inherent uncertainties. Therefore, the CONTAM Panel performed different scenarios by calculating the hypothetical human dietary exposure considering the RPA of 1.0 µg/kg as a maximum occurrence value in meat and meat products, fish and fish products, eggs and egg products and honey consumed as such or as part of composite dishes. This is in agreement with the request to evaluate if the RPA is low enough to protect human health. These scenarios represent highly unlikely situations in which all considered foods of animal origin are contaminated with one nitrofuran marker metabolite. This introduces substantial uncertainty in the estimations of potential human exposure. SEM not only is a marker metabolite of nitrofurazone, but can also originate from several other sources. Except for carrageenan, which is authorised for use as a food additive in a variety of food products, these other sources have been eliminated owing to changes in legislation or are covered by potential occurrence in foods of animal origin, as considered in scenario 1A. Therefore, the CONTAM Panel, in addition to the scenarios which cover foods of animal origin only, calculated the potential chronic dietary exposure to SEM taking into consideration those food categories which may contain carrageenan as an additional potential source of contamination, considered in scenarios 2A and 2C. Depending on the assumed concentration of SEM in carrageenan or the final food, these estimations introduce a substantial uncertainty in the exposure estimations.

In humans, there is potential for additional non-dietary exposure to certain nitrofurans from licensed medicines via oral or topical administration; however, the extent of this additional exposure is not known.

For some nitrofurans, and especially marker metabolites, few, if any, toxicity studies were available. In most cases, the CONTAM Panel used data from toxicity studies on parent compounds to derive reference points for the marker metabolites. Metabolites of the parent compounds, other than the marker metabolites, may also be responsible for toxic effects observed in toxicity studies on parent compounds. The Panel assumes equal potency and equal bioavailability of the nitrofurans and their marker metabolites. The BMDL for carcinogenic effects of furazolidone in rats was potentially underestimated because of the increased mortality at higher doses. There were some indications that furaltadone has a higher potency than furazolidone in inducing mammary tumours in rats, but data were too limited to draw a conclusion.

The CONTAM Panel assumed that the carcinogenicity of furazolidone could be caused by AOZ, because of its genotoxic effects *in vitro* and *in vivo*. However, in view of the similar carcinogenic potencies of furaltadone and furazolidone, the apparent lack of genotoxicity of AMOZ may cast some doubt on the role of AOZ in the carcinogenicity of furazolidone. For the genotoxicity of several marker metabolites, results from *in vitro* studies only are available and, therefore, there is uncertainty regarding the genotoxicity *in vivo*.

Overall, the CONTAM Panel considered that the impact of the uncertainties on the risk assessment of human exposure to nitrofurans and their metabolites through the consumption of food is substantial. The approach taken is more likely to overestimate than underestimate the risk.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

General

- Nitrofurans are synthetic broad spectrum antimicrobial agents. The nitrofurans considered in this opinion are furazolidone, furaltadone, nitrofurantoin, nitrofurazone and nifursol.
- Nitrofurans share a nitrofuran ring which is coupled to a side-chain via an azomethine bond. The side-chains differ for the various drugs, being 3-amino-2-oxazolidinone (AOZ) for furazolidone, 3-amino-5-methylmorpholino-2-oxazolidinone (AMOZ) for furaltadone, 1-aminohydantoin (AHD) for nitrofurantoin, semicarbazide (SEM) for nitrofurazone, and 3,5-dinitrosalicylic acid hydrazide (DNSH) for nifursol.
- In veterinary medicine, nitrofurans are no longer authorised for use in food-producing animals in the EU. In human medicine, furazolidone, nitrofurantoin and nitrofurazone are still used.
- Nitrofurans have short half-lives in animals and, therefore, they do not occur generally as residues in foods of animal origin. Reactive metabolites are formed that are able to bind covalently to tissue macromolecules, such as proteins and DNA. When animal tissues are consumed as food, the side-chains may be released, namely AOZ, AMOZ, AHD, SEM and DNSH.

Methods of analysis

- Because of the short half-lives of the parent nitrofurans, analytical methods have been developed to test for the presence of covalently bound metabolites which have relatively long half-lives.
- The side-chains of the covalently bound residues are used as marker metabolites.
- Generally, both screening and confirmatory methods for the nitrofuran marker metabolites AOZ, AMOZ, AHD, SEM and DNSH in foods of animal origin use acid hydrolysis and nitrobenzaldehyde derivatisation of the released marker metabolites.
- Screening for the resulting nitrophenyl derivatives is generally undertaken by enzyme-linked immunosorbent assays or biosensor methods, providing sufficient analytical sensitivity to meet the current minimum required performance limit (MRPL) of 1 µg/kg.
- Confirmatory methods are based on liquid chromatography–tandem mass spectrometry (LC-MS/MS) and also adequately meet the MRPL of 1 µg/kg.

Appropriateness of using marker metabolites of nitrofurans

- Nitrofuran parent compounds can only be detected in animal tissues and products for a short period after treatment of the animals and, therefore, monitoring of nitrofuran residues in livestock based on the identification of the parent compounds is not appropriate.
- Metabolites binding covalently to proteins and persisting for several weeks in edible tissues, from which the side-chains AOZ, AMOZ, AHD, SEM and DNSH may be released, serve as excellent marker metabolites for the illicit use of nitrofurans in food-producing animals.
- As other nitrofuran metabolites that persist at higher concentrations have not been identified, the marker metabolites AOZ, AMOZ, AHD, SEM and DNSH are appropriate for the reference point for action (RPA) for foods of animal origin.

Occurrence/exposure

- Illicit treatment of food producing animals with nitrofurans may result initially in levels of marker metabolites at the mg/kg level in edible products.
- Data on occurrence of nitrofuran metabolites (AOZ, AMOZ, AHD and SEM) in food, reported by Member States from the National Residue Monitoring Plans, have been extracted for the period 2002 to 2013; there were 214 non-compliant targeted samples reported for nitrofurans over that 12 year period. The categories in which nitrofurans were reported in decreasing level of incidence were poultry, bovines, sheep/goats, pigs, farmed game, honey, rabbit, aquaculture, horses and wild game.
- Data were extracted also from the Rapid Alert System for Food and Feed (RASFF) database for the years 2002 to 2014; there were 808 notification events reported for nitrofuran metabolites (AOZ, AMOZ, AHD and SEM), of which 416 were for crustaceans and products thereof and 150 were for poultry meat and poultry meat products.
- In the last decade, the number of non-compliant samples has decreased, as indicated by the national residue monitoring plans and the RASFF notifications. Most of the non-compliant samples concern AOZ and SEM in poultry, bovine, sheep and goats, and crustaceans.
- The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) concluded that data extracted from the European Commission database and the RASFF database were too limited to carry out a reliable human dietary exposure assessment.
- The CONTAM Panel calculated the hypothetical human dietary exposure considering the occurrence of one nitrofuran marker metabolite at an occurrence value equal to the RPA of 1 µg/kg, for two scenarios (1A and 1B). These scenarios represent worst-case situations, in which all considered foods covered by each scenario are contaminated with one nitrofuran marker metabolite at the level of the RPA, a highly unlikely situation.
- Exposure scenario 1A represents the occurrence of nitrofuran marker metabolites owing to illicit nitrofuran use. In this scenario, foods of animal origin, excluding milk and dairy products, are considered to contain one nitrofuran marker metabolite at the concentration level of 1 µg/kg. The mean chronic dietary exposure across dietary surveys would range from 1.9 to 4.3 ng/kg b.w. per day for adults and would be the highest for toddlers (3.3 to 8.0 ng/kg body weight (b.w.) per day).
- Besides arising from nitrofurazone use, SEM may occur in food from other sources, including use of the food additive carrageenan. The CONTAM Panel considered scenarios (2A-2D) covering the different sources.
- Exposure scenario 2C covers all potential dietary exposure to SEM. This scenario includes foods of animal origin (including only those milk and dairy products for which carrageenan is authorised as an additive) and foods of non-animal origin for which carrageenan is authorised as an additive. These foods are considered to be contaminated with SEM at a concentration equal to the RPA level of 1 µg/kg. The mean chronic dietary exposure to SEM across dietary surveys would range from 6.4 to 16 ng/kg b.w. per day for adults and would be the highest for toddlers (17 to 55 ng/kg b.w. per day).

Hazard identification and characterisation

Toxicokinetics

- Reduction of the nitro-group seems to be the most important metabolic pathway potentially leading to reactive intermediates that are capable of binding to proteins and to DNA. Various other metabolites may be formed in this pathway, including the open-chain cyano-metabolite. Apart from the marker metabolites, very few metabolites have been detected as residues in tissues of treated animals.

- Nitroreduction and subsequent redox cycling results in the generation of reactive species (including oxygen species) that might be responsible for some of the adverse effects.
- Thiol-containing compounds such as glutathione play a role in detoxification of some nitrofuran metabolites, but glutathione adducts have been detected *in vitro* only and seem to be rather unstable owing to a retro-Michael reaction (exchange with other thiol group-containing compounds).
- Based on studies with radiolabelled nitrofurans, high levels (mg/kg range) of metabolites are present in tissues shortly after the last treatment. A proportion of the metabolites cannot be extracted from the tissues with organic solvents and are assumed to be protein-bound. Levels of these residues decrease gradually but are still detectable after 45 days in muscle, kidney and liver of treated pigs and probably for much longer. The decrease of residues in liver and kidney is faster than in muscle tissue.
- As has been shown in different animal species, the side-chains can be released from a proportion of the residues after acid treatment (leading to cleavage of the azomethine bond).
- Feeding of rats with protein-bound residues of radiolabelled furazolidone showed that some of the radiolabel was excreted in urine and so must have been absorbed in the gastrointestinal tract. The radiolabel was also detected in tissues of rats and was partly non-extractable. AOZ could be released by acid treatment from these non-extractable residues in rat tissues.
- Free AOZ was detected in plasma of pigs treated with furazolidone, showing that release of AOZ from furazolidone occurs in these animals, suggesting that acid hydrolysis in the stomach may be an important metabolic pathway for nitrofurans.
- Free AOZ was detected in the blood of rats fed with meat containing only protein-bound residues of furazolidone, showing that AOZ can also be released from these residues, probably in the stomach at low pH.

Toxicity studies

- Acute toxicity studies in laboratory animals showed that for furazolidone, nitrofurantoin and nitrofurazone the lung is an important target for toxicity, leading to decreased respiratory function and death. Signs of neurotoxicity such as hyperirritability, tremors and convulsions were also found.

Furazolidone and AOZ

- For AOZ, hepatotoxicity, decreased body weight gain and anaemia were observed in repeated-dose toxicity studies at the lowest tested dose of 0.9 mg/kg b.w. per day in rats and at 1 mg/kg b.w. per day in dogs.
- Furazolidone in mice was embryotoxic at the lowest dose tested of 200 mg/kg b.w. per day and caused decreased body weight and viability of pups after birth, but no malformations were found.
- Furazolidone and its marker metabolite AOZ are genotoxic *in vitro* and possibly also *in vivo*. As AOZ can be released from bound residues of furazolidone metabolites, these bound residues should be considered genotoxic.
- Furazolidone induced malignant mammary tumours in rats, bronchial adenocarcinomas in male and female mice and neural astrocytomas in male rats. The CONTAM Panel concluded that furazolidone is carcinogenic in mice and rats. No information on the carcinogenicity of AOZ, the marker metabolite of furazolidone, was identified, but it is presumed that AOZ may play a role in tumour formation.

Furaltadone and AMOZ

- Furaltadone is a bacterial and mammalian cell mutagen *in vitro*. The marker metabolite AMOZ is not genotoxic *in vitro*.
- Furaltadone induced malignant mammary tumours in female rats. The CONTAM Panel concluded that furaltadone is carcinogenic in rats. There is no information on the chronic toxicity or the carcinogenicity of AMOZ.

Nitrofurantoin and AHD

- Nitrofurantoin caused toxic effects in liver, kidney and testes, necrosis of the ovarian follicles, decreased weight gain and neurotoxicity in repeated-dose toxicity studies, with a NOAEL of about 120 mg/kg b.w. per day in rats and mice.
- Furazolidone, furaltadone, nitrofurantoin and nitrofurazone caused toxic effects on the testes in rats and mice, but no NOAEL could be identified. Effects were observed at the lowest dose tested of 10 mg/kg b.w. per day for nitrofurantoin.
- Nitrofurantoin was embryotoxic in mice and rats and caused decreased body weight and viability of pups after birth. A NOAEL of 10 mg/kg b.w. per day was identified for embryotoxicity in rats. Malformations were found in offspring of rats and rabbits, with a NOAEL of 30 mg/kg b.w. per day.
- Nitrofurantoin caused peripheral nerve damage in rats treated orally at the lowest dose tested of 20 mg/kg b.w. per day.
- *In vitro*, nitrofurantoin induces mutations, chromosomal aberrations and DNA damage. *In vivo*, it induces DNA damage in multiple organs, micronuclei formation in mice and gene mutations in a transgenic mouse mutation assay. For AHD, the only *in vivo* mutagenicity study which is available shows a negative result.
- Nitrofurantoin induced an increase mainly in benign tumours in mice and rats, but in male rats a few malignant tumours were found. Based on these observations, the CONTAM Panel concluded that there is limited evidence that nitrofurantoin is carcinogenic in rats. No information on the chronic toxicity or the carcinogenicity of AHD was identified.

Nitrofurazone and SEM

- Nitrofurazone caused toxic effects in liver, kidney and testes, decreased weight gain and neurotoxicity in repeated-dose toxicity studies. The NOAEL for effects on the testes in rats was 13.5 mg/kg b.w per day. SEM caused severe deformation of limbs and osteochondral lesions at the lowest tested dose of 23 mg/kg b.w. per day in rats.
- Nitrofurazone was not teratogenic in mice and rabbits at doses that were not maternotoxic. For fetotoxicity/maternotoxicity an overall NOAEL of 14 mg/kg b.w. per day was identified.
- For SEM, in a study looking at the incidence of cleft palate and resorptions only, an effect was found when rats were treated orally with SEM at 25 mg/kg b.w. per day or higher, but not when treated at 10 mg/kg b.w. per day.
- Nitrofurazone showed reproductive toxicity in mice at the lowest dose tested (14 mg/kg b.w. per day).
- SEM caused neurobehavioural effects in juvenile rats when treated orally at the lowest dose tested of 40 mg/kg b.w. per day for 10 days.
- Nitrofurazone and its marker metabolite SEM are genotoxic *in vitro*. *In vivo* tests gave negative results with nitrofurazone, whereas no conclusion can be drawn on the *in vivo* genotoxicity of SEM.

- Nitrofurazone increased the incidence of mainly benign tumours in mice and rats following oral administration. In male rats, a non-dose-related increase in carcinomas of the preputial gland was observed. The CONTAM Panel concluded that there is no evidence for the carcinogenicity of nitrofurazone in mice, and that evidence for its carcinogenicity in rats is equivocal. Non-neoplastic effects of nitrofurazone were observed in a chronic toxicity study at the lowest dose tested of 14 mg/kg b.w. per day in mice (ovarian atrophy in females and reduced survival in males) and the lowest dose tested of about 11 mg/kg b.w. per day in rats (testes degeneration).
- SEM increased the incidence of malignant lung tumours, particularly in female mice. In rats, no increase in tumour incidence was found. The CONTAM Panel concluded that there is limited evidence that SEM is carcinogenic in mice, but not in rats. Based on effects on bones observed in a chronic toxicity study in male rats, a NOAEL of 0.6 mg/kg per day was derived for non-neoplastic effects of SEM.

Nifursol and DNSH

- From a 13-week study in which nifursol caused slight changes in red blood cell parameters, a NOAEL of about 14 mg/kg b.w. per day was identified.
- Nifursol did not have any effects on reproduction in rats treated for three generations at doses of 54 mg/kg b.w. per day or lower.
- Nifursol is genotoxic *in vitro*, whereas *in vivo* it induced neither chromosomal aberrations nor mutations.
- For nifursol the available chronic toxicity studies in rats and dogs did not show clear indication for carcinogenicity. The toxicological information was too limited to derive a NOAEL for non-neoplastic effects of nifursol. No information on the chronic toxicity or the carcinogenicity of DNSH was identified.

Mode of action

- Reduction of the nitro-group seems to be the key metabolic pathway leading to reactive intermediates, including reactive oxygen species. Reactive metabolites are capable of binding to proteins and to DNA, being thereby responsible for most of the adverse effects resulting from exposure to nitrofurans.
- With the exception of AOZ, no information was identified regarding the mode of action of the nitrofuran marker metabolites.
- AOZ plays a role in the inhibition of monoamine-oxidase in animals treated with furazolidone. This may result in an increased susceptibility to neurotoxic effects of certain biogenic amines such as tyramine.
- Protein binding of reactive nitrofuran metabolites may play a role in the irreversible inhibition of the pyruvate dehydrogenase complex, another potential mechanism underlying neurotoxic effects of nitrofurans, such as polyneuritis.

Human data

- The oral administration of furazolidone and nitrofurantoin in humans may lead to a range of adverse reactions, particularly nausea, vomiting and abdominal pain. Both drugs have also been associated with haemolytic anaemia observed in patients deficient in glucose-6-phosphate dehydrogenase.
- The topical use of nitrofurazone may lead to allergic reactions.
- Epidemiological studies are reported only for patients treated with nitrofurantoin, and associations were found for cancers of the nervous system in adults, for drug-induced liver injury and for increased risk of pulmonary adverse events in patients with renal impairment.

Considerations for derivation of a health-based guidance value

- Because furazolidone is genotoxic and carcinogenic, the derivation of an health-based guidance value (HBGV) is not appropriate. A $BMDL_{10}$ value for bronchial adenocarcinomas in mice of 3.5 mg/kg b.w. per day (1.6 mg/kg b.w. per day, expressed as AOZ) was selected as a reference point for carcinogenic effects.
- Non-neoplastic effects of furazolidone and AOZ were found on red blood cell parameters and enzymes in blood. The lowest BMDL was estimated for the effect of AOZ on ALP ($BMDL_{05}$ of 0.02 mg/kg b.w. per day). The CONTAM Panel concluded that this value can be used as a reference point for the risk characterisation for non-neoplastic effects.
- Furaltadone is genotoxic and carcinogenic and therefore the derivation of an HBGV is not appropriate. The CONTAM Panel concluded that the available data do not provide a suitable basis for deriving a reference point. For AMOZ, there is no information on its carcinogenicity, and the limited available data indicate that it is non-genotoxic *in vitro*. Therefore, the CONTAM Panel concluded that the risk of its carcinogenicity cannot be assessed.
- There is no information on non-neoplastic effects of furaltadone or AMOZ that could be used for the derivation of a reference point.
- Nitrofurantoin is genotoxic *in vivo*. Based on the limited evidence for its carcinogenicity, the CONTAM Panel concluded that the compound should be considered genotoxic and carcinogenic. Thus, the derivation of an HBGV for nitrofurantoin is not appropriate. A $BMDL_{10}$ value for osteosarcomas in male rats of 61 mg/kg b.w. per day (29.5 mg/kg b.w. per day, expressed as AHD) was selected as a reference point for carcinogenic effects.
- For non-neoplastic effects, the most sensitive endpoint for nitrofurantoin is impaired spermatogenesis, but the available data did not allow for a BMD analysis or the derivation of a NOAEL. Effects were observed at the lowest dose tested of 10 mg/kg b.w. per day (4.8 mg/kg b.w. per day, expressed as AHD) and this was selected as a reference point for non-neoplastic effects. The CONTAM Panel noted that the effects at this dose are substantial.
- Nitrofurazone is genotoxic *in vitro* but no conclusion could be drawn on its genotoxicity *in vivo* and carcinogenicity. For SEM, which is genotoxic *in vitro*, the CONTAM Panel concluded that it is carcinogenic in mice, but the available information was not suitable to derive a reference point.
- Non-neoplastic effects of nitrofurazone were found on the testes and the epididymis in rats, while, for SEM, effects on bone development were observed. The lowest BMDL was estimated for the effect of SEM on bone development ($BMDL_{10}$ of 1.0 mg/kg b.w.). The CONTAM Panel concluded that this value can be used as a reference point for the risk characterisation for non-neoplastic effects.
- For nifursol, there is no clear indication that it is carcinogenic; it is genotoxic *in vitro* but not *in vivo*. For DNSH, there is no information on its mutagenicity/genotoxicity or carcinogenicity.
- For non-neoplastic effects, a $BMDL_{05}$ value for the effect on liver weight of 11 mg/kg b.w. per day (7.3 mg/kg b.w. per day, expressed as DNSH) was selected as a reference point.

Risk characterisation

- As different critical effects are observed for the different marker metabolites, the CONTAM Panel characterised the risk for each marker metabolite separately.
- For the actual exposure to nitrofuran marker metabolites, no reliable human dietary exposure assessment could be carried out and, therefore, the CONTAM Panel could not characterise the risk.

Evaluation whether a reference point for action of 1 µg/kg for nitrofuran metabolites as defined in the legislation in food of animal origin is adequate to protect public health.

- For AOZ, considering exposure scenario 1A, median chronic dietary exposure across dietary surveys for the average consumer would result in a margin of exposure (MOE) for carcinogenicity of about 2.9×10^5 for toddlers and 6.2×10^5 for adults, and an MOE for non-neoplastic effects of about 3.6×10^3 for toddlers and 7.7×10^3 for adults. The CONTAM Panel considered that, for AOZ, these MOEs for carcinogenicity and non-neoplastic effects are sufficiently large and do not indicate a health concern.
- The CONTAM Panel could not conclude on the carcinogenicity of the furaltadone marker metabolite AMOZ. Given that there are no clear indications that furaltadone is more potent than furazolidone with respect to the induction of mammary adenocarcinomas, the CONTAM Panel concluded that the cancer risk of AMOZ, if any, would not be greater than that from AOZ and hence does not indicate a health concern. The CONTAM Panel could not identify a reference point for non-neoplastic effects for AMOZ.
- For AHD, considering exposure scenario 1A, median chronic dietary exposure across dietary surveys for the average consumer would result in an MOE for carcinogenicity of about 5.4×10^6 for toddlers and 1.1×10^7 for adults and an MOE for non-neoplastic effects of about 8.7×10^5 for toddlers and 1.8×10^6 for adults. The CONTAM Panel considered that, for AHD, these MOEs for carcinogenicity and non-neoplastic effects are sufficiently large and do not indicate a health concern.
- For SEM, the cancer risk could not be assessed. Considering exposure scenario 1A, median chronic dietary exposure across dietary surveys for the average consumer would result in an MOE for non-neoplastic effects of about 1.8×10^5 for toddlers and 3.8×10^5 for adults. The CONTAM Panel considered that, for SEM, these MOEs for non-neoplastic effects are sufficiently large and do not indicate a health concern.
- For DNSH, considering exposure scenario 1A, median chronic dietary exposure across dietary surveys for the average consumer would result in an MOE for non-neoplastic effects of about 1.3×10^6 for toddlers and 2.8×10^6 for adults. The CONTAM Panel considered that, for DNSH, these MOEs for non-neoplastic effects are sufficiently large and do not indicate a health concern.

Assessment of the appropriateness of applying the reference point for action that is considered adequate to protect public health to other commodities than food of animal origin.

- AOZ, AMOZ, AHD and DNSH have not been reported to occur in foods of non-animal origin. Only SEM is reported to occur in foods of non-animal origin owing to its potential presence in the food additive carrageenan, which is used in a large variety of foods. The food additive carrageenan may also be used in foods of animal origin.
- For SEM, the cancer risk could not be assessed. Considering exposure scenario 2C, median chronic dietary exposure across dietary surveys for the average consumer would result in an MOE for non-neoplastic effects of about 3.4×10^4 for toddlers and 1.0×10^5 for adults. The CONTAM Panel considered that, for SEM, these MOEs for non-neoplastic effects are sufficiently large and do not indicate a health concern.

RECOMMENDATIONS

- There is a need for a carcinogenicity study on SEM conducted in accordance with the current guidelines. There is also a need for information on the mechanisms underlying the genotoxic and carcinogenic effects of SEM.

DOCUMENTATION PROVIDED TO EFSA

1. Original study reports submitted to WHO for the risk assessment of furazolidone by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1992 that were made available by the data owner.

Halliday RP, Sutton ML, Sigler FW and Levin RA, 1973a. Chronic toxicopathological safety study (two years) of NF-180 in rats. Unpublished final report project no 475.09-C d.d. 9 November 1973. Pathological and Toxicology Section, Norwich Pharmacal Company, Norwich, New York, NY, USA, not published.

Halliday RP, Sutton ML and Sigler FW, 1973b. Tumorgenesis evaluation (lifetime) of NF-180 in Sprague-Dawley and Fischer 344 rats. Unpublished interim report no 2 project no 475.09D d.d. 9 November 1973. Part I: Sprague-Dawley evaluation. Pathological and Toxicology Section, Norwich Pharmacal Company, Norwich, New York, NY, USA, not published.

Halliday RP, Sutton ML and Sigler FW, 1974. Tumorgenesis evaluation (twenty-three months) of furazolidone (NF-180) in mice. Unpublished final report project no 475.09E d.d. 31 January 1974. Pathological and Toxicology Section, Research and Development Department, Norwich Pharmacal Company, Norwich, New York, NY, USA, not published.

King CD, Sutton ML and Levin RA 1972a. Chronic toxicopathological safety study (two years) of NF-180 in rats. Unpublished status report no 1 project no 475.09c d.d. 18 October 1972. Pathological and Toxicology Section, Research and Development Department, Norwich Pharmacal Company, Norwich, New York, NY, USA, not published.

King CD, Sutton ML, Wong LCK and Laughlin PJ, 1972b. Tumorgenesis evaluation (two years) of NF-180 in Spraque-Dawley and Fischer 344 rats. Unpublished status report no 1 project no 475.09D d.d. 18 0ctober 1972. Pathological and Toxicology Section, Research and Development Department, Norwich Pharmacal Company, Norwich, New York, NY, USA, not published.

Mitchell JM, Trimmer JE, Areia D and Wessel JL, 1990a. Acute oral toxicity study of furazolidone in rats. Unpublished report d.d. 5 July 1991 from project no 5884-90. Bio/Dynamics Inc., East Millstone, NJ, USA, not published.

Mitchell JM, Trimmer JE, Areia D and Wessel JL, 1990b. Acute oral toxicity study of furazolidone in mice. Unpublished report d.d. 5 July 1991 from project no. 5885-90. Bio/Dynamics Inc., East Millstone, NJ, USA, not published.

Siedler AJ and Searfoss W, 1966. Effects of long-term feeding of various nitrofurans to rats. Unpublished report problem no 440.07 d.d. 13 October 1966. Division of Chemotherapy, Research and Development Department, Norwich Pharmacal Company, Norwich, New York, NY, USA. Submitted to WHO by SmithKline Beecham Animal Health, West Chester, PA, USA, not published.

Siedler AJ and Searfoss W, 1967. Effects of long-term feeding of various nitrofurans to rats. Unpublished report problem no 440.07 d.d. 4 January 1976. Division of Chemotherapy, Research and Developmental Department, Norwich Pharmacal Company, Norwich, New York, NY, USA. Submitted to WHO by SmithKline Beecham Animal Health, West Chester, PA, USA, not published.

2. Letter from Marinalg International received on 11 February 2015 in reply to EFSA's request for updated information on the formation and occurrence of semicarbazide in seaweeds used as a food additive.

3. Original study report submitted to EFSA for the risk assessment of semicarbazide in food by the AFC Panel in 2005, which was made available by the data owner.

CTL (Central Toxicology Laboratory), 2004. Semicarbazide: *in vivo* mouse liver unscheduled DNA synthesis assay. CTL study no SM1207, not published.

4. Original study reports submitted to EFSA for the risk assessment of semicarbazide in food by the AFC Panel in 2005, which was made available by the data owner.

Herbold B, 2003. Semicarbazide hydrochloride—*Salmonella*/microsome test plate incorporation method. Bayer HealthCare Study no T 5072934, not published.

Herbold B, 2004. Semicarbazide hydrochloride. *In vitro* chromosome aberration test with Chinese hamster V79 cells. Bayer HealthCare Study no AT01020, not published.

5. Original study reports submitted to the European Commission for the risk assessment of nifursol by the SCAN in 2001 and 2003, which were made available by the data owner.

Allen JA and Proudlock RJ, 1987. Micronucleus test on nifursol. Company report, HRC Report No PDR 455/878, Huntingdon Research Centre, Huntingdon, UK, not published.

Allen JA, Proudlock RJ and Birt DM, 1987. Analysis of metaphase chromosomes obtained from bone marrow of rats treated with nifursol. Company report, HRC Report No PDR 458/87564, Huntingdon Research Centre, Huntingdon, UK, not published.

Ballantyne M, 2003. Nifursol®: evaluation of the possible induction of lacZ- mutations in tissues of treated MutaTMMice, not published.

Benford DJ, 1987a. Nifursol: unscheduled DNA synthesis in hepatocytes and intestinal cells following oral exposure of rats. Report No 10/87/TX, Robens Institute, University of Surrey, Guildford, UK, not published.

Benford DJ, 1987b. Intestinal irritation by nifursol. Report No 18/87/TX, Robens Institute, University of Surrey, Guildford, UK, not published.

Brüll LP, 2003. Determination of Nifursol® and metabolites in turkey skin, muscle, kidney and liver using LC-TIS/MS/MS: depletion study sample analysis, not published.

Cavagnaro J and Cortina T, 1985a. *In vitro* chromosomal aberrations in Chinese hamster ovary cells with nifursol. Company report, Hazleton Report No 186-110, not published.

Cavagnaro J and Cortina T, 1985b. *In vitro* chromosomal aberrations in Chinese hamster ovary cells with nifursol (repeat test). Company report, Hazleton Report No 186-110, not published.

Cavagnaro J and McCarrol NE, 1985. *Salmonella typhimurium*/mammalian microsome plate incorporation assay with compound nifursol. Company report, Hazleton Report No 186-107, not published.

Cavagnaro J and Sernau RC, 1985. Final report: unscheduled DNA synthesis rat hepatocyte assay. Company report, Hazleton Report No 186–109, not published.

Connelly J, 1988. Investigation of binding of nifursol to rat tissue DNA *in vivo*. Report No 11/87/TX, Robens Institute, University of Surrey, Guildford, UK, not published.

Dawes RLF, 1988. Nifursol—analysis of benign tumours. Company report, Internal Document No 56645/86/88, Duphar B.V., Weesp, Netherlands, not published.

George GM, Frahm LJ and McDonnell JP, 1973. Depletion of nifursol residues in turkey tissue from birds medicated with 75 ppm nifursol. Report No TR-37473. Pharmaceutical Development and Analysis Department, Salsbury Laboratories, Charles City, IA, USA, not published.

Green SI, 1980. Mutagenicity testing of nifursol using Ames' test system as performed by M+E consultants, 35 Dean Hill, Plymouth PL9 9AF, dated Jan 1980. Company report, Wickham Laboratories, Wickham, UK, not published.

Jorgenson TA, 1967. Three generation reproduction study in rats. Company report, Project No 145, Test No RRT-35-67. Salsbury Laboratories, Research Division, Charles City, IA, not published.

Kan CA, 2003. A Nifursol® depletion study in turkeys, not published.

Lozano JA and Morrison JL, undated. The metabolism of nifursol (3,5-dinitrosalicylic acid 5-nitrofurfurylidene hydrazine) in the turkey and rat. Company report, Salsbury Laboratories, Research Division, Charles City, IA, USA, not published.

Rude TA, 1970b. Pathology associated with a chronic oral toxicity test of nifursol given continuously in the feed of dogs for two years. Company report, Project No 145, Test No DCT-3567. Salsbury Laboratories, Research Division, Charles City, IA, USA, not published.

Rude TA, 1970c. Rat chronic toxicity: chronic oral toxicity study of nifursol given continuously in the feed of rats for two years and 3 months (27 months). Company report, Project No 145, Test No RCT-3567. Salsbury Laboratories, Research Division, Charles City, IA, USA, not published.

van Kolfschoten, 1988. Review of the genotoxic potential of nifursol. Company report, Internal Document No 56645/74/88, Duphar B.V., Weesp, Netherlands, not published.

Wood JD, Coleman M, Heywood R, Street AE, Gopinath C, Jolly DW, Gibson WA and Anderson A, 1984. Nifursol toxicity to rats by continuous dietary administration for 13 weeks followed by a 4-week withdrawal period (final report). Company report, HRC Report No SLY 3/84961, Huntingdon Research Centre, Huntingdon, UK, not published.

6. Original study reports submitted to EMA for the risk assessment of furazolidone by the Committee for Veterinary Medicinal Products (CVMP) in 1995, which were made available by the data owner.

Brinck P, Damm Jørgensen K and Skydsgaard M, 1995. 3-amino-oxazolidinone-2, 3-month oral (dietary) toxicity study in the dog, not published.

de Groot AJL and van Zeeland AA, 1994. Evaluation of the formation of DNA adducts by 3-amino-oxazolidinone-2 in male mouse liver (*in vivo* assay), not published.

NOTOX, 1994a. Assessment of acute oral toxicity with 3-amino-5(4-morphomethyl)-2-oxazolidinon in the rat. NOTOX project 107696, not published.

NOTOX, 1994b. Assessment of acute oral toxicity with 3-amino-oxazolidinone-2 in the rat. NOTOX project 107663 22/04/1994, not published.

NOTOX, 1994c. Evaluation of the ability of 3-amino-5(4-morphomethyl)-2-oxazolidinon to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat) NOTOX project 107685, not published.

NOTOX, 1994d. Evaluation of the ability of 3-amino-oxazolidinone-2 to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat) NOTOX project 107652, not published.

NOTOX, 1994e. Evaluation of the mutagenic activity of 3-amino-5(4-morphomethyl)-2-oxazolidinon in the Ames *Salmonella*/microsome test and the *Escherichia coli*/microsome test (with independent repeat). NOTOX project 107674, not published.

NOTOX, 1994f. Evaluation of the mutagenic activity of 3-amino-oxazolidinone-2 in the Ames *Salmonella*/microsome test and the *Escherichia coli*/microsome test (with independent repeat) NOTOX project 107641, not published.

NOTOX, 1994g. Micronucleus test in bone marrow cells of the mouse with 3-amino-oxazolidinone-2. Notox project 127057, not published.

NOTOX, 1994h. Micronucleus test in bone marrow cells of the mouse with 3-amino-oxazolidinone-2. Notox project 130196, not published.

NOTOX, 1995a. 14-day dietary dose range finding study with 3-amino-oxazolidinone-2 in the rat. Notox project 129307, not published.

NOTOX, 1995b. 90-day dietary toxicity study with 3-amino-oxazolidinone-2 in the rat. NOTOX project 129757, not published.

RCC NOTOX B.V., 1990a. Evaluation of the mutagenic activity of furaltadone hydrochloride in an *in vitro* mammalian cell gene mutation test with L5178Y mouse lymphoma cells (with independent repeat) RCC NOTOX project 059039, not published.

RCC NOTOX B.V., 1990b. Evaluation of the mutagenic activity of furazolidone in an *in vitro* mammalian cell gene mutation test with L5178Y mouse lymphoma cells (with independent repeat) RCC NOTOX project 059028, not published.

7. Data on usage levels of carrageenan (E407). Submitted to EFSA by nine data providers.

REFERENCES

Abraham RT, Knapp JE, Minnigh MB, Wong LK, Zemaitis MA and Alvin JD, 1984. Reductive metabolism of furazolidone by *Escherichia coli* and rat liver *in vitro*. Drug Metabolism and Disposition, 12, 732–741.

Abramsson-Zetterberg L and Svensson K, 2005. Semicarbazide is not genotoxic in the flow cytometry-based micronucleus assay *in vivo*. Toxicology Letters, 155, 211–217.

Adkison KK, Vaidya SS, Lee DY, Koo SH, Li L, Mehta AA, Gross AS, Polli JW, Lou Y and Lee EJ, 2008. The ABCG2 C421A polymorphism does not affect oral nitrofurantoin pharmacokinetics in healthy Chinese male subjects. British Journal of Clinical Pharmacology, 66, 233–239.

Akao M, Kuroda K, Kanisawa M and Miyaki K, 1971. Influence of some nitrofurans on carcinogenesis in rats fed 4-(dimethylamino) azobenzene. GANN Japanese Journal of Cancer Research, 62, 479–484.

Akinshina LP, Tanirbergenov TB, Bobrinev EV and Zolotareva GN, 1992. Genotoxicity of 5-nitrofuran derived medicines in 2 bacterial short-term assays. Genetika, 28, 89–98.

Altamirano A and Bondani A, 1989. Adverse reactions to furazolidone and other drugs. A comparative review. Scandinavian Journal of Gastroenterology 169 (Suppl.), 70–80.

An H, Henry M, Cain T, Tran B, Paek HC and Farley D, 2012. Determination of total nitrofuran metabolites in shrimp muscle using liquid chromatography/tandem mass spectrometry in the atmospheric pressure chemical ionization mode. Journal of AOAC International, 95, 1222–1233.

Anderson D and Phillips BJ, 1985. Nitrofurazone–genotoxicity studies in mammalian cels *in vitro* and *in vivo*. Food and Chemical Toxicology, 23, 1091–1098.

Aperia AC and Liebow AA, 1964. Implications of urine PO_2 for renal medullary blood flow. American Journal of Physiology, 206, 499–504.

Aracena P, Lazo-Hernandez C, Molina-Berrios A, Sepulveda DR, Reinoso C, Larrain JI, Navarro J and Letelier ME, 2014. Microsomal oxidative stress induced by NADPH is inhibited by nitrofurantoin redox biotranformation. Free Radical Research, 48, 129–136.

Ardsoongnearn C, Boonbanlu O, Kittijaruwattana S and Suntornsuk L, 2014. Liquid chromatography and ion trap mass spectrometry for simultaneous and multiclass analysis of antimicrobial residues in feed water. Journal of Chromatography B, 945, 31–38.

Ask K, Décologne N, Asare N, Holme JA, Artur Y, Pelczar H and Camus P, 2004. Distribution of nitroreductive activity toward nilutamide in rat. Toxicology and Applied Pharmacology, 201, 1–9.

Aufrère MB, Hoener BA and Vore M, 1978. Reductive metabolism of nitrofurantoin in the rat. Drug Metabolism and Disposition, 6, 403–411.

Babile R, Queinnec G, Berland HM and Darre R, 1978. Chromosome structure of pig lymphocytes and food additives. Comptes Rendus des Seances de la Société de Biologie et de ses Filiales, 172, 546–553.

Bajaj AK and Gupta SC, 1986. Contact Hypersensitivity to Topical Antibacterial Agents. International Journal of Dermatology, 25, 103–105.

Barbosa J, Ferreira ML, Ramos F and da Silveira MIN, 2007a. Determination of the furaltadone metabolite 5-methylmorpholino-3-amino-2-oxazolidinone (AMOZ) using liquid chromatography coupled to electrospray tandem mass spectrometry during the nitrofuran crisis in Portugal. Accreditation and Quality Assurance, 12, 543–551.

Barbosa J, Moura S, Barbosa R, Ramos F and da Silveira MIN, 2007b. Determination of nitrofurans in animal feeds by liquid chromatography-UV photodiode array detection and liquid chromatography-ionspray tandem mass spectrometry. Analytica Chimica Acta, 586, 359–365.

Barbosa J, Freitas A, Moura S, Mourão JL, Noronha da Silveira MI and Ramos F, 2011. Detection, accumulation, distribution, and depletion of furaltadone and nifursol residues in poultry muscle, liver, and gizzard. Journal of Agricultural and Food Chemistry, 59, 11927–11934.

Barbosa J, Freitas A, Mourão JL, Noronha da Silveira MI and Ramos F, 2012. Determination of furaltadone and nifursol residues in poultry eggs by liquid chromatography–electrospray ionization tandem mass spectrometry. Journal of Agricultural and Food Chemistry, 60, 4227–4234.

Barragán Hernández EÁ, Herrera Montalvo LA, Ocampo Camberos L and Sumano López H, 2011. Genotoxicidad de la furazolidona y la forma libre de su metabolito 3-amino-2-oxazolidona, mediante la prueba de micronúcleos en linfocitos humanos. Veterinaria México, 42, 289–298.

Becalski A, Lau BP-Y, Lewis D and Seaman SW, 2004. Semicarbazide formation in azodicarbonamide-treated flour: a model study. Journal of Agricultural and Food Chemistry, 52, 5730–5734.

Behar A, Rachmilewitz E, Rahamimoff R and Denman M, 1965. Experimental nitrofurantoin polyneuropathy in rats. Early histological and electro physiological alterations in peripheral nerves. Archives of Neurology, 13, 160–163.

Bellomonte G, Filesi C, Macri A, Mosca M and Sanzini E, 1993. High performance liquid chromatographic determination of nitrofurans and free chloramphenicol in poultry muscle, liver and eggs. Italian Journal of Food Science, 5, 247–253.

Bertenyi KK and Lambert IB, 1996. The mutational specificity of furazolidone in the *lacI* gene of *Escherichia coli*. Mutation Research, 357, 199–208.

BgVV (Bundesinstitut fuer gesundheitlichen Verbraucherschutz und Veterinaermedizin), 2002. Gesundheitliche Bewertung von Nitrofuranen in Lebensmitteln. Stellungnahme des BgVV, 1–3.

Bignami M, Morpurgo G, Pagliani R, Carere A, Conti G and Di Giuseppe G, 1974. Non-disjunction and crossing-over induced by pharmaceutical drugs in *Aspergillus nidulans*. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 26, 159–170.

Björnsson E, Talwalkar J, Treeprasertsuk S, Kamath PS, Takahashi N, Sanderson S, Neuhauser M and Lindor K, 2010. Drug-induced autoimmune hepatitis: clinical characteristics and prognosis. Hepatology, 51, 2040–2048.

Bock C, Stachel C and Gowik P, 2007. Validation of a confirmatory method for the determination of residues of four nitrofurans in egg by liquid chromatography-tandem mass spectrometry with the software InterVal. Analytica Chimica Acta, 586, 348–358.

Boelsterli UA, Ho HK, Zhou S and Leow KY, 2006. Bioactivation and hepatotoxicity of nitroaromatic drugs. Current Drug Metabolism, 7, 715–727.

Borghoff SJ, Prescott JS, Janszen DB, Wong BA and Everitt JI, 2001. Alpha 2u-Globulin nephropathy, renal cell proliferation, and dosimetry of inhaled tert-butyl alcohol in male and female F-344 rats. Toxicological Sciences, 61, 176–186.

Borroto JIG, Machado GP, Creus A and Marcos R, 2005. Comparative genotoxic evaluation of 2-furylethylenes and 5-nitrofurans by using the comet assay in TK6 cells. Mutagenesis 20, 193–197.

Borsa M, Bulletti GM and Zoni G, 1976. Nitrofurantoin: dimensions of crystals and biologic availability. Bollettino chimico farmaceutico, 115, 483–488

Botsoglou NA and Fletouris DJ (eds), 2001. Drug residues in foods: pharmacology, food safety, and analysis. CRC Press, Taylor & Francis Group, London, 1149 pp.

Böttiger LE and Westerholm B, 1977. Adverse drug reactions during treatment of urinary tract infections. European Journal of Clinical Pharmacology, 11, 439–442.

Boyd MR, Stiko AW and Sasame HA, 1979. Metabolic activation of nitrofurantoin–possible implications for carcinogenesis. Biochemical Pharmacology, 28, 601–606.

Brander GC, Pugh DM, Bywater RJ and Jenkins WL, 1991. Veterinary applied pharmacology and therapeutics. Baillière Tindall, London, 624 pp.

Brayfield A (ed.), 2014. Martindale: The complete drug reference. 38th Edition. Pharmaceutical Press, London, UK, 4688 pp.

Brumfitt W and Hamilton-Miller JM, 1998. Efficacy and safety profile of long-term nitrofurantoin in urinary infections: 18 years' experience. Journal of Antimicrobial Chemotherapy, 42, 363–371.

Butler WH, Graham TC and Sutton ML, 1990a. Chronic toxicity and oncogenicity studies of macrodantin in Sprague-Dawley rats. Food and Chemical Toxicology, 28, 269–277.

Butler WH, Graham TC and Sutton ML, 1990b. Oncogenicity study of macrodantin in Swiss mice. Food and Chemical Toxicology, 28, 49–54.

Buzard JA and Conklin JD, 1964. Placental transfer of nitrofurantoin and furaltadone. American Journal of Physiology, 206, 189–192.

Buzard JA, Conklin JD, O'Keefe E and Paul MF, 1961. Studies on the absorption, distribution and elemination of nitrofurantoin in the rat. Journal of Pharmacology and Experimental Therapeutics, 131, 38–43.

Buzard JA, Paul MF and Vrablic DM, 1956. Colorimetric determination of nitrofurazone, nitrofurantoin, and furazolidone in plasma. Antibiotics and Chemotherapy, 6, 702–707.

Calafatti SA, Ortiz RA, Deguer M, Martinez M and Pedrazzoli J Jr, 2001. Effect of acid secretion blockade by omeprazole on the relative bioavailability of orally administered furazolidone in healthy volunteers. British Journal of Clinical Pharmacology, 52, 205–209.

Callen DF, 1981. Comparison of the genetic activity of AF-2 and nitrofurantoin in log and stationary phase cells of saccharomyces cerevisiae. Environmental Mutagenesis, 3, 651–658.

Carlsson Forslund L, 2014. Risk evaluation of nitrofurans in animal food products. Degree project in biology, Biology Education Centre, Uppsala University, and the Swedish National Food Agency, 109 pp.

Chadfield MS and Hinton MH, 2003. Evaluation of treatment and prophylaxis with nitrofurans and comparison with alternative antimicrobial agents in experimental *Salmonella enterica* Serovar enteritidis infection in chicks. Veterinary Research Communications, 27, 257–273.

Chadseesuwan U, Puthong S, Gajanandana O, Palaga T and Komolpis K, 2013. Development of an enzyme-linked immunosorbent assay for 1-aminohydantoin detection. Journal of AOAC International, 96, 680–686.

Chalasani N, Fontana RJ, Bonkovsky HL, Watkins PB, Davern T, Serrano J, Yang H and Rochon J, 2008. Causes, clinical features, and outcomes from a prospective study of drug-induced liver injury in the United States. Gastroenterology, 135, 1924–1934.

Chang C, Peng D-P, Wu J-E, Wang Y-L and Yuan Z-H, 2008. Development of an indirect competitive ELISA for the detection of furazolidone marker residue in animal edible tissues. Journal of Agricultural and Food Chemistry, 56, 1525–1531.

Chatterjee SN, Banerjee SK, Pal AK and Basak J, 1983. DNA damage, prophage induction and mutation by furazolidone. Chemico-Biological Interactions, 45, 315–326.

Cheng CC, Hsieh KH, Lei YC, Tai YT, Chang TH, Sheu SY, Li WR and Kuo TF, 2009. Development and residue screening of the furazolidone metabolite, 3-amino-2-oxazolidinone (AOZ), in cultured fish by an enzyme-linked immunosorbent assay. Journal of Agricultural and Food Chemistry, 57, 5687–5692.

Cheng KC, Cahill DS, Kasai H, NishimuraS and Loeb LA, 1992. 8-hydroxyguanine, an abundant form of oxidative DNA damage, causes G-> T and A -> C substitutions. Journal of Biological chemistry, 267, 166–172.

Chu P-S and Lopez MI 2005 Liquid chromatography-tandem mass spectrometry for the determination of protein-bound residues in shrimp dosed with nitrofurans. Journal of Agricultural and Food Chemistry, 53, 8934–8939.

Chu P-S, Lopez MI, Abraham A, El Said KR and Plakas SM, 2008. Residue depletion of nitrofuran drugs and their tissue-bound metabolites in channel catfish (*Ictalurus punctatus*) after oral dosing. Journal of Agricultural and Food Chemistry, 56, 8030–8034.

Chu P-S and Lopez MI, 2007. Determination of nitrofuran residues in milk of dairy cows using liquid chromatography-tandem mass spectrometry. Journal of Agricultural and Food Chemistry, 55, 2129–2135.

Chumanee S, Sutthivaiyakit S and Sutthivaiyakit P, 2009. New reagent for trace determination of protein-bound metabolites of nitrofurans in shrimp using liquid chromatography with diode array detector. Journal of Agricultural and Food Chemistry, 57, 1752–1759.

Cohen MM and Sagi M, 1979. The effect of nitrofurans on mitosis, chromosome breakage and sister-chromatid exchanges in human peripheral lymphocytes. Mutation Research, 59, 139–142.

Cohen SM, Ertűrk F, Van Esch AM, Crovetti AJ and Bryan GT, 1973. Carcinogenicity of 5-nitrofurans, 5-nitroimidazoles, 4-nitrobenzenes and related compounds. Journal of the National Cancer Institute, 51, 403–417.

Conklin JD and Hailey FJ, 1969. Urinary drug excretion in man during oral dosage of different nitrofurantoin formulations. Clinical Pharmacology and Therapeutics, 10, 534-539.

Conneely A, Nugent A, O'Keeffe M, Mulder PPJ, van Rhijn JA, Kovacsics L, Fodor A, McCracken RJ and Kennedy DG, 2003. Isolation of bound residues of nitrofuran drugs from tissue by solid-phase extraction with determination by liquid chromatography with UV and tandem mass spectrometric detection. Analytica Chimica Acta, 483, 91–98.

Cooper KM and Kennedy DG, 2005. Nitrofuran antibiotic metabolites detected at parts per million concentrations in retina of pigs—a new matrix for enhanced monitoring of nitrofuran abuse. Analyst, 130, 466–468.

Cooper KM and Kennedy DG, 2007. Stability studies of the metabolites of nitrofuran antibiotics during storage and cooking. Food Additives and Contaminants, 24, 935–942.

Cooper KM, Elliott CT and Kennedy DG, 2004. Detection of 3-amino-2-oxazolidinone (AOZ), a tissue-bound metabolite of the nitrofuran furazolidone, in prawn tissue by enzyme immunoassay. Food Additives and Contaminants, 21, 841–848.

Cooper K, Mulder PJ, Van Rhijn J, Kovacsics L, McCracken R, Young P and Kennedy D, 2005. Depletion of four nitrofuran antibiotics and their tissue-bound metabolites in porcine tissues and determination using LC-MS/MS and HPLC-UV. Food Additives and Contaminants, 22, 406–414.

Cooper KM, Le J, Kane C and Kennedy DG, 2008a. Kinetics of semicarbazide and nitrofurazone in chicken eggs and egg powders. Food Additives and Contaminants, 25, 684–692.

Cooper KM, McCracken RJ, Buurman M and Kennedy DG, 2008b. Residues of nitrofuran antibiotic parent compounds and metabolites in eyes of broiler chickens. Food Additives and Contaminants, 25, 548–556.

Cooper KM, Samsonova JV, Plumpton L, Elliott CT and Kennedy DG, 2007. Enzyime immunoassay for semicarbazide – The nitrufuran metabolite and food contaminant. Analytica Chimica Acta, 592, 64–71.

Coraggio MJ, Gross TP and Roscelli JD, 1989. Nitrofurantoin toxicity in children. Pediatric Infectious Disease Journal, 8, 163–166.

Crews C, 2014. Potential natural sources of semicarbazide in honey. Journal of Apicultural Research, 53, 129–140.

Cunha BA, 1988. Nitrofurantoin—current concepts. Urology, 32, 67–71.

Czeizel AE, Rockenbauer M, Sorensen HT and Olsen J, 2001. Nitrofurantoin and congenital abnormalities. European Journal of Obstetrics, Gynecology, and Reproductive Biology, 95, 119–126.

Dalvie DK, Kalgutkar AS, Khojasteh-Bakht SC, Obach RS and O'Donnell JP, 2002. Biotransformation reactions of five-membered aromatic heterocyclic rings. Chemical Research in Toxicology, 15, 269–99.

D'Arcy PF, 1985. Nitrofurantoin. Drug Intelligence and Clinical Pharmacy, 19, 540–547.

De Angelis I, Rossi L, Pedersen JZ, Vignoli AL, Vincentini O, Hoogenboom LA, Polman TH, Stammati A and Zucco F, 1999. Metabolism of furazolidone: alternative pathways and modes of toxicity in different cell lines. Xenobiotica, 29, 1157–1169.

De Flora S, 1979. Metabolic activation and deactivation of mutagens and carcinogens. Italian Journal of Biochemistry, 28, 81–103.

De Flora S, 1981. Study of 106 organic and inorganic compounds in the Salmonella/microsome test. Carcinogenesis, 2, 283–298.

De Flora S, Zanacchi P, Camoirano A, Bennicelli C and Badolati GS, 1984. Genotoxic activity and potency of 135 compounds in the Ames reversion test and in a bacterial DNA-repair test. Mutation Research, 133, 161–198.

De la Calle MB and Anklam E, 2005. Semicarbazide: occurrence in food products and state-of-the-art in analytical methods used for its determination. Analytical and Bioanalytical chemistry, 382, 968–977.

De la Calle MB and Szilagyi S, 2006. Determination of semicarbazide in fresh egg and whole egg powder by liquid chromatography/tandem mass spectrometry: interlaboratory validation study. Journal of AOAC International, 89, 1664–1671.

de la Fuente del Rey M, 1986. Teratogenic effect of semicarbazide in Wistar rats. Biology of the Neonate, 49, 150–157.

de la Fuente M, Hernanz A and Alia M, 1983a. Effect of semicarbazide on the surfactant phospholipid percentages during fetal and postnatal lung development in rat. Comparative Biochemistry and Physiology C, 74, 115–118.

de la Fuente M, Hernanz A and Alia M, 1983b. Effect of semicarbazide on the perinatal development of the rat: changes in DNA, RNA and protein content. Methods and Findings in Experimental and Clinical Pharmacology, 5, 287–297.

de la Fuente M, Hernanz A and Alia M, 1983c. Effects of semicarbazide on DNA, RNA and protein hepatic levels on four subsequent generations of Wistar rats. Comparative Biochemistry and Physiology C, 74, 447–450.

Degroodt JM, De Bukanski BW, De Groof J, Beernaert H and Srebrnk S, 1992. Chloramphenicol and nitrofuran residue analysis by HPLC and photodiode array detection in meat and fish. Journal of Liquid Chromatography, 15, 2355–2371.

Diblikova I, Cooper KM, Kennedy DG and Franek M, 2005. Monoclonal antibody-based ELISA for the quantification of nitrofuran metabolite 3-amino-2-oxazolidinone in tissues using a simplified sample preparation. Analytica Chimica Acta, 540, 285–292.

Dimitrieska-Stojkovic E, Arsova G, Hajrulai-Musliu Z, Stojanovska-Dimzoska B, Uzunov R, Todorovic A and Stojkovic G, 2012. In-house validation and quality control of commercial enzyme-linked immunosorbnet assays for screening of nitrofuran metabolites in food of animal origin. Macedonian Veterinary Review, 35, 13–21.

Dodd MC, 1946. The chemotherapeutic properties of 5-nitro-2-furaldehyde semicarbazone (furacin). Journal of Pharmacology and Experimental Therapeutics, 86, 311–323.

Douny C, Widart J, De Pauw E, Silvestre F, Kestemont P, Tu HT, Phuong NT, Maghuin-Rogister G and Scippo M-L, 2013. Development of an analytical method to detect metabolites of nitrofurans: application to the study of furazolidone elimination in Vietnamese black tiger shrimp (*Penaeus monodon*). Aquaculture, 376, 54–58.

Du NN, Chen MM, Sheng LQ, Chen SS, Xu HJ, Liu ZD, Song CF and Qiao R, 2014. Determination of nitrofuran metabolites in shrimp by high performance liquid chromatography with fluorescence detection and liquid chromatography-tandem mass spectrometry using a new derivatization reagent. Journal of Chromatography A, 31, 90–96.

Edder P, Vargas S, Ortelli D and Corvi C, 2003. Analysis of nitrofuran metabolites in food by high-performance liquid chromatography with tandem mass spectrometry detection. Clinical Chemistry and Laboratory Medicine, 41, 1608–1614.

EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to semicarbazide in food. The EFSA Journal 2005, 219, 1–36.

EFSA (European Food Safety Authority), 2009. Guidance of the Scientific Committee on a request from EFSA on the use of the benchmark dose approach in risk assessment. The EFSA Journal 2009, 1150, 1–72.

EFSA (European Food Safety Authority), 2011a. Evaluation of the FoodEx, the food classification system applied to the development of the EFSA Comprehensive European Food Consumption Database. EFSA Journal 2011;9(3):1970, 22 pp. doi:10.9203/j.efsa.2011.1970

EFSA (European Food Safety Authority), 2011b. Use of the EFSA Comprehensive European Food Consumption Database in exposure assessment. EFSA Journal 2011;9(3):2097, 34 pp. doi:10.2903/j.efsa.2011.2097

EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2013. Guidance on methodological principles and scientific methods to be taken into account when establishing Reference Points for Action (RPAs) for non-allowed pharmacologically active substances present in food of animal origin. EFSA Journal, 2013;11(4):3195, 24 pp. doi:10.2903/j.efsa.2013.3195

EFSA SC (EFSA Scientific Committee), 2011. Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment. EFSA Journal 2011;9(9):2379, 69 pp. doi:10.2903/j.efsa.2011.2379

EFSA SC (EFSA Scientific Committee), 2012. Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA Journal 2012;10(3):2579, 32 pp. doi:10.2903/j.efsa.2012.2579

EMA (European Agency for the Evaluation of Medicinal Products), 1996. Committee for Veterinary Medicinal Products: Nitrofurans. Summary Report, Veterinary Medicines Evaluation Unit, 1–2.

EMA (European Agency for the Evaluation of Medicinal Products), 1997. Committee for Veterinary Medicinal Products: Furazolidone. Summary Report, Veterinary Medicines Evaluation Unit, 1–3.

Epstein SS, Arnold E, Andrea J, Bass W and Bishop Y, 1972. Detection of chemical mutagens by the dominant lethal assay in the mouse. Toxicology and Applied Pharmacology, 23, 288–325.

Ertürk E, Morr JE, Cohen SM, Price JM and Bryan GT, 1970. Transplantable rat mammary tumors induced by 5-nitro-2-furaldehyde semicarbazone and by formic acid 2-[4-(5-nitro-2-furyl)–2-thiazolyl] hydrazide. Cancer Research, 30, 1409–1412.

FAO (Food and Agriculture Organization of the United Nations), 2005. Fishery Information Data and Statistics Unit. FISHSTAT+Databases and Statistics. Rome, Italy, Food and Agriculture Organization of the United Nations.

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization), 1993a. Toxicological evaluation of certain veterinary drug residues in food. Furazolidone. WHO Food Additives Series, 31.

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization), 1993b. Toxicological evaluation of certain veterinary drug residues in food. Nitrofural (nitrofurazone). WHO Food Additives Series, 31.

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization), 1993c. Evaluation of certain veterinary drug residues in food. Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives. Furazolidone. WHO Technical Report Series, 832, 32–37.

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization), 1993d. Evaluation of certain veterinary drug residues in food. Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives. Nitrofural (nitrofurazone). WHO Technical Report Series, 832, 37–40.

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization), 1993e. Residues of some veterinary drugs in animals and foods. Furazolidone. FAO Food and Nutrition paper, 41/5, 87–103.

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization), 1993f. Residues of some veterinary drugs in animals and foods. Nitrofurazone. FAO Food and Nutrition paper, 41/5, 105–112.

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization), 2009. Principles and methods for the risk assessment of chemicals in food. Environmental Health Criteria 240.

Fau D, Berson A, Eugene D, Fromenty B, Fisch C and Pessayre D, 1992. Mechanism for the hepatotoxicity of the antiandrogen, nilutamide. Evidence suggesting that redox cycling of this nitroaromatic drug leads to oxidative stress in isolated hepatocytes. Journal of Pharmacology and Experimental Therapeutics, 263, 69–77.

Finzi JK, Donato JL, Sucupira M and De Nucci G, 2005. Determination of nitrofuran metabolites in poultry muscle and eggs by liquid chromatography-tandem mass spectrometry. Journal of Chromatography B, 824, 30–35.

Fonatsch C, 1977. Effect of nitrofurantoin on meiosis of the male mouse. Human Genetics, 39, 345–351.

Franek M, Diblikova I, Vass M, Kotkova L, Stastny K, Frgalova K and Hruska K, 2006. Validation of a monoclonal antibody-based ELISA for the quantification of the furazolidone metabolite (AOZ) in eggs using various sample preparation. Veterinarni Medicina, 51, 248–257.

FSA (Food Standards Agency), 2002. Results of the Department of Agriculture and Rural Development testing programme for nitrofuran in chicken sampled from cold stores. FSA, London, UK.

FSANZ (Food Standards Australia New Zealand), 2004. Nitrofurans in prawns. A toxicological review and risk assessment. Technical Report Series No 31. Available at: http://www.foodstandards.gov.au/publications/documents/31_Nitrofurans%20in%20prawns_edit.pdf

Fucić A, Marković D, Ferencić Z, Mildner B, Jazbec AM and Spoljar JB, 2005. Comparison of genomic damage caused by 5-nitrofurantoin in young and adult mice using the *in vivo* micronucleus assay. Environmental and Molecular Mutagenesis, 46, 59–63.

Gao N, Ni YC, Thornton-Manning JR, Fu PP and Heflich RH, 1989. Mutagenicity of nitrofurantoin and furazolidone in Chinese hamster ovary cell strains. Mutation Research, 225, 181–187.

Geerts AF, Eppenga WL, Heerdink R, Derijks HJ, Wensing MJ, Egberts TC and De Smet PA, 2013. Ineffectiveness and adverse events of nitrofurantoin in women with urinary tract infection and renal impairment in primary care. European Journal of Clinical Pharmacology, 69, 1701–1707.

George JD, Fail PA, Grizzle TB and Heindel JJ, 1996. Nitrofurazone: reproductive assessment by continuous breeding in Swiss mice. Fundamental and Applied Toxicology, 34, 56–66.

Gerk PM, Kuhn RJ, Desai NS and McNamara PJ, 2001. Active transport of nitrofurantoin into human milk. Pharmacotherapy, 21, 669–675.

Gerk PM, Moscow JA and McNamara PJ, 2003. Basolateral active uptake of nitrofurantoin in the CIT3 cell culture model of lactation. Drug Metabolism and Disposition, 31, 691–693.

Glascock HW Jr, MacLeod PF, Davis JB, Cuddihy RV and Anzlowar BR, 1969. Is nitrofurazone a primary irritant or a potent sensitizer? A review of the literature, 1945–1965, and cases reported to the medical director. Review of Allergy, 23, 52–58.

Gleckman R, Alvarez S and Joubert DW, 1979. Drug therapy reviews: nitrofurantoin. American Journal of Hospital Pharmacy, 36, 342–351.

Goldenthal EI, 1971. A compilation of LD_{50} values in newborn and adult animals. Toxicology and Applied Pharmacology, 18, 185–207.

Goodman DR, Hakkinen PJ, Nemenzo JH and Vore M, 1977. Mutagenic evaluation of nitrofuran derivatives in *Salmonella typhimurium*, by the micronucleus test, and by *in vivo* cytogenetics. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 48, 295–305.

Gottschall DW and Wang R, 1995. Depletion and bioavailability of [^{14}C]furazolidone residues in swine tissues. Journal of Agricultural and Food Chemistry, 43, 2520–2525.

Grave K, Engelstad M, Søli NE and Håstein T, 1990. Utilization of antibacterial drugs in salmonid farming in Norway during 1980–1988. Aquaculture, 86, 347–358.

Grave K, Markestad A and Bangen M, 1996. Comparison in prescribing patterns of antibacterial drugs in salmonid farming in Norway during the periods 1980–1988 and 1989–1994. Journal of Veterinary Pharmacology and Therapeutics, 19, 184–191.

Green MHL, Rogers AM, Muriel WJ, Ward AC and McCalla DR, 1977. Use of a simplified fluctuation test to detect and characterize mutagenesis by nitrofurans. Mutation Research, 44, 139–143.

Guinebault PR, Broquaire M and Theboult JJ, 1981. Comptes-redus proceedings. Technique et Documentation. Proceedings of the First European Congress of Biopharmacy and Pharmacokinetics, Paris, France, 151–158.

Guo JJ, Chou HN and Chiu Liao I, 2003. Disposition of 3-(4-cyano-2-oxobutylidene amino)-2-oxazolidone, a cyano-metabolite of furazolidone, in furazolidone-treated grouper. Food Additives and Contaminants, 20, 229–236.

Hagenäs L, Ploen L and Ritzen EM, 1978. The effect of nitrofurazone on the endocrine, secretory and spermatogenic functions of the rat testis. Andrologia, 10, 107–126.

Hardin BD, Schuler RL, Burg JR, Booth GM, Hazelden KP, MacKenzie KM, Piccirillo VJ and Smith KN, 1987. Evaluation of 60 chemicals in a preliminary developmental toxicity test. Teratogenesis, Carcinogenesis, and Mutagenesis, 7, 29–48.

Heaton LH and Post G, 1968. Tissue residue and oral safety of furazolidone in four species of trout. The Progressive Fish-Culturist, 30, 208–215.

Hirakawa K, Midorikawa K, Oikawa S, Kawanishi S, 2003. Carcinogenic semicarbazide induces sequence-specific DNA damage through the generation of reactive oxygen species and the derived organic radicals. Mutation Research 536 (2003) 91–101.

Hiraku Y, Sekine A, Nabeshi H, Midorikawa K, Murata M, Kumagai Y and Kawanishi S, 2004. Mechanism of carcinogenesis induced by a veterinary antimicrobial drug, nitrofurazone, via oxidative DNA damage and cell proliferation. Cancer Letters, 215, 141-150.

Hoener B and Patterson SE, 1981. Nitrofurantoin disposition. Clinical Pharmacology and Therapeutics, 29, 808–816.

Hoener BA, 1988. Nitrofurazone: kinetics and oxidative stress in the singlepass isolated perfused rat liver. Biochemical Pharmacology, 37, 1629–1636.

Hoenicke K, Gatermann R, Hartig L, Mandix M and Otte S, 2004. Formation of semicarbazide (SEM) in food by hypochlorite treatment: is SEM a specific marker for nitrofurazone abuse? Food Additives and Contaminants, 21, 526–537.

Holmberg L and Boman G, 1981. Pulmonary reactions to nitrofurantoin. 447 cases reported to the Swedish Adverse Drug Reaction Committee 1966–1976. European Journal of Respiratory Diseases, 62, 180–189.

Holmberg L, Boman G, Bottiger LE, Eriksson B, Spross R and Wessling A, 1980. Adverse reactions to nitrofurantoin. Analysis of 921 reports. American Journal of Medicine, 69, 733–738.

Hoogenboom LAP and Polman THG, 1993. Simultaneous detection of protein bound residues of the nitrofuran drugs furazolidone, furaltadone, nitrofurazone and nitrofurantoin. Proceedings of the Residues of Veterinary Drugs in Food: EuroResidue II Conference, Utrecht, Netherlands, 376–381.

Hoogenboom LA, Oorsprong MB, van Vliet T and Kuiper HA, 1991a. The use of pig hepatocytes for cytotoxicity studies of veterinary drugs: a comparative study with furazolidone and other nitrofurans. Toxicology In Vitro, 5, 31–38.

Hoogenboom LA, Tomassini O, Oorsprong MB and Kuiper HA, 1991b. Use of pig hepatocytes to study the inhibition of monoamine oxidase by furazolidone. Food and Chemical Toxicology, 29, 185–191.

Hoogenboom LA, van Kammen M, Berghmans MC, Koeman JH and Kuiper HA, 1991c. The use of pig hepatocytes to study the nature of protein-bound metabolites of furazolidone: a new analytical method for their detection. Food and Chemical Toxicology, 29, 321–328.

Hoogenboom LA, Berghmans MC, Polman TH, Parker R and Shaw IC, 1992a. Depletion of protein-bound furazolidone metabolites containing the 3-amino-2-oxazolidinone side-chain from liver, kidney and muscle tissues from pigs. Food Additives and Contaminants, 9, 623–630.

Hoogenboom LA, van Kammen M, Huveneers-Oorsprong MB and Kuiper HA, 1992b. Study on the role of glutathione in the biotransformation and toxicity of furazolidone using pig hepatocytes. Toxicology In Vitro, 6, 227–237.

Hoogenboom LA, Polman TH, Lommen A, Huveneers MB and van Rhijn J, 1994. Biotransformation of furaltadone by pig hepatocytes and *Salmonella typhimurium* TA 100 bacteria, and the formation of protein-bound metabolites. Xenobiotica, 24, 713–727.

Hoogenboom LA, van Bruchem GD, Sonne K, Enninga IC, van Rhijn JA, Heskamp H, Huveneers-Oorsprong MB, van der Hoeven JC and Kuiper HA, 2002. Absorption of a mutagenic metabolite released from protein-bound residues of furazolidone. Environmental Toxicology and Pharmacology, 11, 273–287.

Horne E, Cadogan A, O'Keeffe M and Hoogenboom LA, 1996. Analysis of protein-bound metabolites of furazolidone and furaltadone in pig liver by high-performance liquid chromatography and liquid chromatography-mass spectrometry. Analyst, 121, 1463–1468.

Hossain M, Ahmed S, Rahman M, Kamaruzzaman B, Jalal K and Amin S, 2013. Method development and validation of nitrofuran metabolites in shrimp by liquid chromatographic mass spectrometric system. Journal of Biological Sciences, 13.

Hu XZ, Xu Y and Yediler A, 2007. Determinations of residual furazolidone and its metabolite, 3-amino-2-oxazolidinone (AOZ), in fish feeds by HPLC-UV and LC-MS/MS, respectively. Journal of Agricultural and Food Chemistry, 55, 1144–1149.

Huber WG, 1982. Nitrofuran derivatives. In: Veterinary Pharmacology and Therapeutics. Eds Booth NH and McDonald LE. Iowa State University Press, Ames, IA, USA, 767.

Hunt RH, Xiao SD, Megraud F, Leon-Barua R, Bazzoli F, van der Merwe S, Vaz Coelho LG, Fock M, Fedail S, Cohen H, Malfertheiner P, Vakil N, Hamid S, Goh KL, Wong BC, Krabshuis J and Le Mair A, 2011. *Helicobacter pylori* in developing countries. World Gastroenterology Organisation Global Guideline. Journal of Gastrointestinal and Liver Diseases, 20, 299-304.

Hurtaud-Pessel D, Verdon E, Blot J and Sanders P, 2006. Proficiency study for the determination of nitrofuran metabolites in shrimps. Food Additives and Contaminants, 23, 569–578.

Huybrechts I, Sioen I, Boon PE, Ruprich J, Lafay L, Turrini A, Amiano P, Hirvonen T, De Neve M, Arcella D, Moschandreas J, Westerlund A, Ribas-Barba L, Hilbig A, Papoutsou S, Christensen T, Oltarzewski M, Virtanen S, Rehurkova I, Azpiri M, Sette S, Kersting M, Walkiewicz A, Serra-Majem L, Volatier JL, Trolle E, Tornaritis M, Busk L, Kafatos A, Fabiansson S, De Henauw S and Van Klaveren JD, 2011. Dietary exposure assessments for children in Europe (the EXPOCHI project): rationale, methods and design. Archives of Public Health, 69, 4. doi:10.1186/0778-7367-1169-1184

IARC (International Agency for Research on Cancer), 1974. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man. Vol 7. 5-(Morpholinomethyl)-3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone. IARC Press, Lyon, France, 161–169.

IARC (International Agency for Research on Cancer), 1976. IARC Monographs on the Evaluation of Cercinogenic Risk of Chemicals to Man. Some carbamates, thiocarbamates and carbazides. IARC Press, Lyon, France, 12.

IARC (International Agency for Research on Cancer), 1983. IARC Monograph on the Evaluation of Carcinogenic Risk of Chemicals to Humans. Vol. 31. Furazolidone. IARC Press, Lyon, France. 141–151.

IARC (International Agency for Research on Cancer), 1987. IARC Monographs on the Evaluation of Carcinogenic Risk to Humans. Overall evaluations of carcinogenicity: an updating of IARC monographs volumes 1 to 42, supplement 7. 449 pp.

IARC (International Agency for Research on Cancer), 1990a. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol 50. Nitrofurantoin. IARC Press, Lyon, France, 211–231.

IARC (International Agency for Research on Cancer), 1990b. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 50. Nitrofural (nitrofurazone). IARC Press, Lyon, France. 195–209.

Ito A NM, Naito Y and Watanabe H, 1983. Tumorigenicity test of N-(5-nitro-2-furfurylidene)–1-aminohydantoin by dietary administration in BDF1 mice. Hiroshima Journal of Medicine, 32, 99–102.

Ito K, Ishida K, Takeuchi A, Nii A, Okamiya H and Doi K, 2002. Nitrofurazone induces non-regenerative hepatocyte proliferation in rats. Experimental and Toxicologic Pathology, 53, 421–426.

Ito K, Sasaki S, Yoshida K, Nii A, Okamiya H and Sakai T, 2000. Collaborative work to evalutae toxicity on male reproductive organs by repeated dose studies in rats 20) testicular toxicity of nitrofurazone after 2 and 4 weeks. Journal of Toxicological Sciences, 25, 195–201.

Jackson D and Robson JM, 1957. The action of furazolidone on pregnancy. Journal of Endocrinology, 15, 355–359.

Jenney EH and Pfeiffer CC, 1958. The convulsant effect of hydrazides and the antidotal effect of anticonvulsants and metabolites. Journal of Pharmacology and Experimental Therapeutics, 122, 110–123.

Jester EL, Abraham A, Wang Y, El Said KR and Plakas SM, 2014. Performance evaluation of commercial ELISA kits for screening of furazolidone and furaltadone residues in fish. Food Chemistry, 145, 593–598.

Jia Q, Yu S, Cheng N, Wu L, Jia J, Xue X and Cao W, 2014. Stability of nitrofuran residues during honey processing and nitrofuran removal by macroporous adsorption resins. Food Chemistry, 162, 110–116.

Jin X, Tang S, Chen Q, Zou J, Zhang T, Liu F, Zhang S, Sun C and Xiao X, 2011. Furazolidone induced oxidative DNA damage via up-regulating ROS that caused cell cycle arrest in human hepatoma G2 cells. Toxicology Letters, 201, 205–212.

Jonen HG, Oesch F and Platt KL, 1980. 4-Hydroxylation of nitrofurantoin in the rat. A 3-methylcholanthrene-inducible pathway of a relatively nontoxic compound. Drug Metabolism and Disposition, 8, 446–451.

Kahlson G and Rosengren E, 1959. Prevention of foetal development by enzyme inhibition. Nature, 184, 1238–1239.

Kari FW, Huff JE, Leininger J, Haseman JK and Eustis SL, 1989. Toxicity and carcinogenicity of nitrofurazone in F344/N rats and B6C3F1 mice. Food and Chemical Toxicology, 27, 129–137.

Karpman E and Kurzrock EA, 2004. Adverse reactions of nitrofurantoin, trimethoprim and sulfamethoxazole in children. Journal of Urology, 172, 448–453.

Kaufmann A, Butcher P, Maden K and Widmer M, 2004. LC-MS-MS method for determining nifursol and other nitrofuran residues in meat. Mitteilungen aus Lebensmitteluntersuchung und Hygiene, 95, 135–146.

Kedderis GL and Miwa GT, 1988. The metabolic activation of nitroheterocyclic therapeutic agents. Drug Metabolism Review, 19, 33–62.

Kennedy DG, Young PB and McCraken RJ, 2003. Analysis of veterinary drug residues in food: the nitrofuran issue. Mitteilungen aus Lebensmitteluntersuchung und Hygiene, 94, 510–526.

Khong SP, Gremaud E, Richoz J, Delatour T, Guy PA, Stadler RH and Mottier P, 2004. Analysis of matrix-bound nitrofuran residues in worldwide-originated honeys by isotope dilution high-performance liquid chromatography-tandem mass spectrometry. Journal of Agricultural and Food Chemistry, 52, 5309–5315.

Kijima A, Ishii Y, Takasu S, Matsushita K, Kuroda K, Hibi D, Suzuki Y, Nohmi T and Umemura T, 2015. Chemical structure-related mechanisms underlying *in vivo* genotoxicity induced by nitrofurantoin and its constituent moieties in gpt delta rats. Toxicology, 331, 125-135.

Klee S, Baumung I, Kluge K, Ungemach FR, Horne E, O'Keeffe M, De Angelis I, Vignoli AL, Zucco F and Stammati A, 1999. A contribution to safety assessment of veterinary drug residues: *in vitro/ex vivo* studies on the intestinal toxicity and transport of covalently bound residues. Xenobiotica, 29, 641–654.

Kramers PG, 1982. Studies on the induction of sex-linked recessive lethal mutations in *Drosophila melanogaster* by nitroheterocyclic compounds. Mutation Research, 101, 209–236.

Krantz JC Jr and Evans WE Jr, 1945. A contribution to the pharmacology of 5-nitro-2-furaldehyde semicarbazone. Journal of Pharmacology and Experimental Therapeutics, 85, 324–331.

Krongpong L, Futami K, Katagiri T, Endo M and Maita M, 2008. Application of ELISA-based kit for detecting AOZ and determining its clearance in eel tissues. Fisheries Science, 74, 1055–1061.

Law FC and Meng J, 1996. Binding of ^{14}C-furazolidone metabolites to the muscular and hepatic proteins of trout. Food Additives and Contaminants, 13, 199–209.

Lax D and Kukolich SG, 1992. Generation of furazolidone radical anion and its inhibition by glutathione. Biochemical Medicine and Metabolic Biology, 48, 56–63.

Leitner A, Zöllner P and Lindner W, 2001. Determination of the metabolites of nitrofuran antibiotics in animal tissue by high-performance liquid chromatography–tandem mass spectrometry. Journal of Chromatography A, 939, 49–58.

Levin RM, Lavkar RM, Monson FC, Witowski BA, Wein AJ, Hanno PM and Ruggieri MR, 1988. Effect of chronic nitrofurantoin on the rabbit urinary bladder. Journal of Urology, 139, 400–404.

Li J, Liu JX and Wang JP, 2009. Multidetermination of four nitrofurans in animal feeds by a sensitive and simple enzyme-linked immunosorbent assay. Journal of Agricultural and Food Chemistry, 57, 2181–2185.

Li J, Liu J, Zhang H-C, Li H and Wang J-P, 2010. Broad specificity indirect competitive immunoassay for determination of nitrofurans in animal feeds. Analytica Chimica Acta, 678, 1–6.

Li Y, Han J, Wang Y, Ma J, Yan Y and Cao L, 2013. Optimization of extraction and determination of chloramphenicol in livestock meat samples using aqueous two-phase system of n-propanol and potassium citrate coupled with HPLC. Journal of the Brazilian Chemical Society, 24, 669–674.

Liao IC, Guo JJ and Su M, 2000. The use of chemicals in aquaculture in Taiwan, Province of China. In: Use of chemicals in aquaculture in Asia. Eds Arthur JR, Lavilla-Pitogo CR Subasinghe RP. Proceedings of the Meeting on the Use of Chemicals in Aquaculture in Asia, Tigbauan, Iloilo, Philippines, 193–205.

Liu W, Kou J, Jiang X, Zhang Z and Qi H, 2012a. Determination of nitrofurans in feeds based on silver nanoparticle-catalyzed chemiluminescence. Journal of Luminescence, 132, 1048–1054.

Liu Y, Huang L, Wang Y, Yang B, Ishan A, Fang K, Peng D, Liu Z, Dai M and Yuan Z, 2010a. Tissue depletion and concentration correlations between edible tissues and biological fluids of 3-amino-2-oxazolidinone in pigs fed with a furazolidone-medicated feed. Journal of Agricultural and Food Chemistry, 58, 6774–6779.

Liu Y, Peng D, Huang L, Wang Y, Chang C, Ihsan A, Tao Y, Yang B and Yuan Z, 2010b. Application of a modified enzyme-linked immunosorbent assay for 3-amino-2-oxazolidinone residue in aquatic animals. Analytica Chimica Acta, 664, 151–157.

Liu YC, Jiang W, Chen YJ, Xiao Y, Shi JL, Qiao YB, Zhang HJ, Li T and Wang Q, 2013. A novel chemiluminescent ELISA for detecting furaltadone metabolite, 3-amino-5-morpholinomethyl-2-oxazolidone (AMOZ) in fish, egg, honey and shrimp samples. Journal of Immunological Methods, 395, 29–36.

Liu Y-T, Ai X-H, Suo W-W, Yu S-M and Yang Q-H, 2012b. Tissue distribution and elimination rules of the metabolite (AOZ) of furazolidone residues in *Ietalurus punetaus*. Freshwater Fisheries, 42, 38–44.

Lopez MI, Feldlaufer MF, Williams AD and Chu PS, 2007. Determination and confirmation of nitrofuran residues in honey using LC-MS/MS. Journal of Agricultural and Food Chemistry, 55, 1103–1108.

Lu C, McCalla DR and Bryant DW, 1979. Action of nitrofurans on *E. coli*: mutation and induction and repair of daughter-strand gaps in DNA. Mutation Research, 67, 133–144.

Luo PJ, Jiang WX, Beier RC, Shen JZ, Jiang HY, Miao H, Zhao YF, Chen X and Wu YN, 2012. Development of an enzyme-linked immunosorbent assay for determination of the furaltadone etabolite, 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ) in animal tissues. Biomedical and Environmental Sciences, 25, 449–457.

Madrigal-Bujaidar E, Ibanez JC, Cassani M and Chamorro G, 1997. Effect of furazolidone on sister-chromatid exchanges, cell proliferation kinetics, and mitotic index *in vivo* and *in vitro*. Journal of Toxicology and Environmental Health, 51, 89–96.

Mandell GL and Sande MA, 1985. Antimicrobial agents. In: The Pharmacological Basis of Therapeutics, ed 7. Eds Gilman AG, Goodman LS, Rall TW and Murad F. MacMillan Publishing Co, New York, USA, 1095–1114.

Männistö P, 1978. The effect of crystal size, gastric content and emptying rate on the absorption of nitrofurantoin in healthy human volunteers. Int J Clin Pharmacol Biopharm, 16, 223–228.

Maranghi F, Tassinari R, Lagatta V, Moracci G, Macri C, Eusepi A, Di Virgilio A, Scattoni ML and Calamandrei G, 2009. Effects of food contaminant semicarbazide following oral administration in juvenile Sprague-Dawley rats. Food and Chemical Toxicology, 47, 472–479.

Maranghi F, Tassinari R, Marcoccia D, Altieri I, Catone T, De Angelis G, Testai E, Mastrangelo S, Evandri MG, Bolle P and Lorenzetti S, 2010. The food contaminant semicarbazide acts as an endocrine disrupter: Evidence from an integrated *in vivo/in vitro* approach. Chemico-Biological Interactions, 183, 40–48

Matsuoka A, Hayashi M and Ishidate M, 1979. Chromosomal aberration tests on 29 chemicals combined with S9 mix *in vitro*. Mutation Research, 66, 277–290.

McCalla DR, Reuvers A and Kaiser C, 1971. Breakage of bacterial DNA by nitrofuran derivatives. Cancer Research, 31, 2184–2188.

McCalla DR and Voutsinos D, 1974. On the mutagenicity of nitrofurans. Mutation Research, 26, 3–16.

McCalla DR, Voutsinos D and Olive PL, 1975. Mutagen screening with bacteria: niridazole and nitrofurans. Mutation Research, 31, 31–37.

McCracken RJ and Kennedy DG, 1997. The bioavailability of residues of the furazolidone metabolite 3-amino-2-oxazolidinone in porcine tissues and the effect of cooking upon residue concentrations. Food Additives and Contaminants, 14, 507–513.

McCracken RJ and Kennedy DG, 2007. Detection, accumulation and distribution of nitrofuran residues in egg yolk, albumen and shell. Food Additives and Contaminants, 24, 26–33.

McCracken RJ, Blanchflower WJ, Rowan C, McCoy MA and Kennedy DG, 1995. Determination of furazolidone in porcine tissue using thermospray liquid chromatography-mass spectrometry and a study of the pharmacokinetics and stability of its residues. Analyst, 120, 2347–2351.

McCracken RJ, McCoy MA and Kennedy DG, 1997. The prevalence and possible causes of bound and extractable residues of the furazolidone metabolite 3-amino-2-oxazolidinone in porcine tissues. Food Additives and Contaminants, 14, 287–294.

McCracken RJ, Spence DE, Floyd SD and Kennedy DG, 2001. Evaluation of the residues of furazolidone and its metabolite, 3-amino-2-oxazolidinone (AOZ), in eggs. Food Additives and Contaminants, 18, 954–959.

McCracken RJ, Van Rhijn JA and Kennedy DG, 2005a. The occurrence of nitrofuran metabolites in the tissues of chickens exposed to very low dietary concentrations of the nitrofurans. Food Additives and Contaminants, 22, 567–572.

McCracken RJ, Van Rhijn JA and Kennedy DG, 2005b. Transfer of nitrofuran residues from parent broiler breeder chickens to broiler progeny. British Poultry Science, 46, 287–292.

McCracken R, Hanna B, Ennis D, Cantley L, Faulkner D and Kennedy DG, 2013. The occurrence of semicarbazide in the meat and shell of Bangladeshi fresh-water shrimp. Food Chemistry, 136, 1562–1567.

McMahon RE, Cline JC and Thompson CZ, 1979. Assay of 855 test chemicals in ten tester strains using a new modification of the Ames test for bacterial mutagens. Cancer Research, 39, 682–693.

Mercado C, Molina F, Navas J, Quinones C and Eylar EH, 1991. Inhibition of T cell mitogenesis by nitrofurans. Biochemical Pharmacology, 41, 503–508.

Merino G, Jonker JW, Wagenaar E, van Herwaarden AE and Schinkel AH, 2005. The breast cancer resistance protein (BCRP/ABCG2) affects pharmacokinetics, hepatobiliary excretion, and milk secretion of the antibiotic nitrofurantoin. Molecular Pharmacology, 67, 1758–1764.

Merino G, Perez M, Real R, Egido E, Prieto JG and Alvarez AI, 2010. *In vivo* inhibition of BCRP/ABCG2 mediated transport of nitrofurantoin by the isoflavones genistein and daidzein: a comparative study in Bcrp1 (-/-) mice. Pharmaceutical Research, 27, 2098–2105.

Miller C, Folkes LK, Mottley C, Wardman P and Mason RP, 2002. Revisiting the interaction of the radical anion metabolite of nitrofurantoin with glutathione. Archives of Biochemistry and Biophysics, 397, 113–118.

Miyaji T, 1971. Acute and chronic toxicity of furylfuramide in rats and mice. Tohoku Journal of Experimental Medicine, 103, 331–369.

Miyaji T, Miyamoto M and Ueda Y, 1964. Inhibition of spermatogenesis and atrophy of the testis caused by nitrofuran compounds. Acta Pathologica Japonica, 14, 261–273.

Montemurro DG, 1960. The effect of nitrofurazone on the testes and accessory sex organs of normal rats and rats bearing the Walker carcinoma 256. British Journal of Cancer, 14, 319–326.

Moreno SN, Mason RP and Docampo R, 1984. Reduction of nifurtimox and nitrofurantoin to free radical metabolites by rat liver mitochondria. Evidence of an outer membrane-located nitroreductase. Journal of Biological Chemistry, 259, 6298–6305.

Mori K, Yasuno A and Matsumoto K, 1960. Induction of pulmonary tumors in mice with isonicotinic acid hydrazid. GANN Japanese Journal of Cancer Research, 51, 83–90.

Morris JE, Price JM, Lalich JJ and Stein RJ, 1969. The carcinogenic activity of some 5-nitrofuran derivatives in the rat. Cancer Research, 29, 2145–2156.

Mottier P, Khong S-P, Gremaud E, Richoz J, Delatour T, Goldmann T and Guy PA, 2005. Quantitative determination of four nitrofuran metabolites in meat by isotope dilution liquid chromatography–electrospray ionisation–tandem mass spectrometry. Journal of Chromatography A, 1067, 85–91.

Muaksang K, 2009. Development of reference methods and reference materials for trace level antibiotic residues in food using IDMS. PhD thesis, University of New South Wales, Sydney, Australia. 303 pp.

Mulder P, Zuidema T, Keestra N, Kooij P, Elbers I and Van Rhijn J, 2005. Determination of nifursol metabolites in poultry muscle and liver tissue. Development and validation of a confirmatory method. Analyst, 130, 763–771.

Murakami E, Ishii J, Muneta S, Hiwada K and Kokubu T, 1989. Blood pressure elevation caused by inhibition of brain glutathione reductase. Journal of Hypertension, 7(Suppl.), S24–S25.

Nesterenko I VE, Filippova K, Komarov A, Panin AN, 2012. Development of a direct competitive ELISA for the detection of furazolidone metabolite in foodstuff of animal origin. Journal of Veterinary Pharmacology and Therapeutics, 35, 91.

Ni YC, Heflich RH, Kadlubar FF and Fu PP, 1987. Mutagenicity of nitrofurans in *Salmonella typhimurium* TA98, TA98NR and TA98/1,8-DNP6. Mutation Research, 192, 15–22.

NIOSH (National Institute for Occupational Safety and Health), 1983. Registry of toxic effects of chemical substances, Vol. 2, as cited by NTP, 1989.

Nishimura T, Aze Y and Ozeki Y, 1995. Effects of nitrofurazone on spermatogenesis and reproductive toxicity in male rats—part of a collaborative work to determine optimal administration period and endpoints. Journal of Toxicological Sciences, 20, 341–349.

Nomura T, Kimura S, Kanzaki T, Tanaka H, Shibata K, Nakajima H, Isa Y, Kurokawa N, Hatanaka T, Kinuta M, Masada K and Sakamote Y, 1984. Induction of tumors and malformations in mice after prenatal treatment with some antibiotic drugs. Medical Journal of Osaka University, 35, 13–17.

Nordeng H, Lupattelli A, Romoren M and Koren G, 2013. Neonatal outcomes after gestational exposure to nitrofurantoin. Obstetrics and Gynecology, 121, 306–313.

Nouws JF and Laurensen J, 1990. Postmortal degradation of furazolidone and furaltadone in edible tissues of calves. Veterinary Quarterly, 12, 56–59.

NTP (National Toxicology Program), 1985. Teratologic evaluation of nitrofurazone (CAS No 59-87-0) administered to CD-1 mice on gestational days 6 through 15. National Toxicology Program/Research Triangle Institute Report NTP-85–335.

NTP (National Toxicology Program), 1987. Teratologic evaluation of nitrofurazone (CAS No 59-87-0) administered to New Zealand white rabbits on gestational days 6 through 19. National Toxicology Program/National Institute of Environmental Health Sciences Report NTP-87–200.

NTP (National Toxicology Program), 1988. Toxicological and carcinogenesis studies of nitrofurazone in F344/N rats and B6C3F1 Mice. National Toxicology Program Technical Report Series No 337.

NTP (National Toxicology Program), 1989. Toxicological and carcinogenesis studies of nitrofurantoin in F344/N rats and B6C3F1 Mice. National Toxicology Program Technical Report Series No 341.

Nuñez-Vergara LJ, Sturm JC, Olea-Azar C, Navarrete-Encina P, Bollo S and Squella JA, 2000. Electrochemical, UV-visible and EPR studies on nitrofurantoin: nitro radical anion generation and its interactions with glutathione. Free Radical Research, 32, 399–409.

O'Keeffe M, Conneely A, Cooper KM, Kennedy DG, Kovacsics L, Fodor A, Mulder PPJ, van Rhijn JA and Trigueros G, 2004. Nitrofuran antibiotic residues in pork: The FoodBRAND retail survey. Analytica Chimica Acta, 520, 125–131.

O'Mahony J, Moloney M, McConnell RI, el Benchikh O, Lowry P, Furey A and Danaher M, 2011. Simultaneous detection of four nitrofuran metabolites in honey using a multiplexing biochip screening assay. Biosensors and Bioelectronics, 26, 4076–4081.

Obaseiki-Ebor EE and Akerele JO, 1986. Nitrofuran mutagenicity: induction of frameshift mutations. Mutation Research Letters, 175, 149–152.

Okura T, Ibe M, Umegaki K, Shinozuka K and Yamada S, 2010. Effects of dietary ingredients on function and expression of P-glycoprotein in human intestinal epithelial cells. Biological & Pharmaceutical Bulletin, 33, 255–259.

Olive PL and McCalla DR, 1975. Damage to mammalian cell DNA by nitrofurans. Cancer Research, 35, 781–784.

Ong TM, 1977. Mutagenic activities of nitrofurans in *Neurospora crassa*. Mutation Research, 56, 13–20.

Oo CY, Paxton EW and McNamara PJ, 2001. Active transport of nitrofurantoin into rat milk. Advances in Experimental Medicine and Biology, 501, 547–552.

Paik SG, 1985. Micronucleus induction in mouse bone marrow cells of some nitrofuran 5 nitroimidazole and nitrothiazole derivatives used as trichomonacides in Korea. Environmental Mutagens and Carcinogens, 5, 61–72.

Pal AK, Rahman MS and Chatterjee SN, 1992. On the induction of *umu* gene expression in *Salmonella typhimurium* strain TA1535/pSK1002 by some nitrofurans. Mutation Research, 280, 67–71.

Palaniyappan V, Nagalingam AK, Ranganathan HP, Kandhikuppam KB, Kothandam HP and Vasu S, 2013. Microwave-assisted derivatisation and LC-MS/MS determination of nitrofuran metabolites in farm-raised prawns (*Penaeus monodon*). Food Additives and Contaminants, 30, 1739–1744.

Palm D, Magnus U, Grobecker H and Jonsson J, 1967. Hemmung der Monoaminoxydase durch bakteriostatisch wirksame Nitrofuran-Derivate. Naunyn-Schmiedebergs Archiv für Pharmakologie und Experimentelle Pathologie, 256, 281–300.

Park YH, Hwang SY, Hong MK and Kwon KH, 2012. Use of antimicrobial agents in aquaculture. Revue scientifique et technique—International Office of Epizootics, 31, 189–197.

Parodi S, Pala M, Russo P, Balbi C, Abelmoschi ML, Taningher M, Zunino A, Ottaggio L, de Ferrari M, Carbone A and Santi L, 1983. Alkaline DNA fragmentation, DNA disentanglement evaluated viscosimetrically and sister chromatid exchanges, after treatment *in vivo* with nitrofurantoin. Chemico-Biological Interactions, 45, 77–94.

Paul HE, Ells VR, Kopko F and Bender RC, 1960a. Metabolic Degradation of the Nitrofurans. Journal of Medicinal and Pharmaceutical Chemistry, 2, 563-584.

Paul HE and Paul MF, 1964. The nitrofurans chemotherapeutic properties. Vol 2, Part 1. Eds Schnitzer RJ and Hawking F. Academic Press, New York, NY, USA, 307–370.

Paul HE, Paul MF and Kopko F, 1952. Effect of furacin (5-nitro-2-furaldehyde semicarbazone) on the *in vitro* metabolism of mammalian tissues. Proceedings of the Society for Experimental Biology and Medicine, 79, 555–558.

Paul HE, Paul MF, Kopko F, Bender RC and Everett G, 1953. Carbohydrate metabolism studies on the testis of rats fed certain nitrofurans. Endocrinology, 53, 585–592.

Paul MF, Bryson MJ and Harrington C, 1956. Effect of furacin on pyruvate metabolism. Journal of Biological Chemistry, 219, 463–471.

Paul MF, Paul HE, Bender RC, Kopko F, Harrington CM, Ells VR and Buzard JA, 1960b. Studies on the distribution and excretion of certain nitrofurans. Antibiotica et Chemotherapia, 10, 287–302.

Pereira AS, Donato JL and De Nucci G, 2004. Implications of the use of semicarbazide as a metabolic target of nitrofurazone contamination in coated products. Food Additives and Contaminants, 21, 63–69.

Pérez M, Real R, Mendoza G, Merino G, Prieto JG and Alvarez AI, 2009. Milk secretion of nitrofurantoin, as a specific BCRP/ABCG2 substrate, in assaf sheep: modulation by isoflavones. Journal of Veterinary Pharmacology and Therapeutics, 32, 498–502.

Perry JE and Leblanc AL, 1967. Transfer of nitrofurantoin across the human placenta. Texas Reports on Biology and Medicine, 25, 265–269.

Peterson FJ, Mason RP, Hovsepian J, Holtzman JL, 1979. Oxygen-sensitive and -insensitive nitroreduction by *Escherichia coli* and rat hepatic microsomes**.** Journal of Biological Chemistry 1979, 254:4009–4014.

Peterson FJ, Combs GF, Holtzman JL and Mason RP, 1982. Effect of selenium and Vitamin E deficiency on nitrofurantoin toxicity in the chick. Journal of Nutrition, 112, 1741–1746.

Pietruszka K, Olejinik M and Sell B, 2007. Development and validation of a liquid chromatography method for the determination of nitrofurans in water. Bulletin of the Veterinary Institute in Pulawy, 51, 267–270.

Pimpitak U, Putong S, Komolpis K, Petsom A and Palaga T, 2009. Development of a monoclonal antibody-based enzyme-linked immunosorbent assay for detection of the furaltadone metabolite, AMOZ, in fortified shrimp samples. Food Chemistry, 116, 785–791.

Plakas SM, el Said KR and Stehly GR, 1994. Furazolidone disposition after intravascular and oral dosing in the channel catfish. Xenobiotica, 24, 1095–1105.

Points J, Thorburn Burns D and Walker MJ, 2015. Forensic issues in the analysis of trace nitrofuran veterinary residues in food of animal origin. Food Control, 50, 92–103.

Polnaszek CF, Peterson FJ, Holtzman JL and Mason RP, 1984. No detectable reaction of the anion radical metabolite of nitrofurans with reduced glutathione or macro-molecules. Chemico-Biological Interactions, 51, 263–271.

Prytherch JP, Sutton ML and Denine EP, 1984. General reproduction, perinatal-postnatal, and teratology studies of nitrofurantoin macrocrystals in rats and rabbits. Journal of Toxicology and Environmental Health, 13, 811–823.

Quillardet P, Arrault X, Michel V and Touati E, 2006. Organ-targeted mutagenicity of nitrofurantoin in Big Blue transgenic mice. Mutagenesis, 21, 305–311.

Radovnikovic A, Moloney M, Byrne P and Danaher M, 2011. Detection of banned nitrofuran metabolites in animal plasma samples using UHPLC–MS/MS. Journal of Chromatography B, 879, 159–166.

Radovnikovic A, Conroy ER, Gibney M, O'Mahony J and Danaher M, 2013. Residue analyses and exposure assessment of the Irish population to nitrofuran metabolites from different food commodities in 2009–2010. Food Additives and Contaminants, 30, 1858–1869.

Rao DN and Mason RP, 1987. Generation of nitro radical anions of some 5-nitrofurans, 2- and 5-nitroimidazoles by norepinephrine, dopamine, and serotonin. A possible mechanism for neurotoxicity caused by nitroheterocyclic drugs. Journal of Biological Chemistry, 262, 11731–11736.

Rascher W and Neubert A, 2012. Reinfektionsprophylaxe rezidivierender Harnwegsinfektionen. Monatsschrift Kinderheilkunde, 160, 171–173.

RIVM (Rijksinstituut voor Volksgezondheid en Milieu), 2003. Advies inzake residuen van furazolidon in garnalen. Project V/320105/01/AA. RIVM, Bilthoven, Netherlands.

Robertson EL, 1982. Antiprotozoan drugs. In: Veterinary pharmacology and therapeutics. Eds Booth NH and McDonald L.E., Iowa State University Press, Ames, IA, USA, 874.

Rodziewicz L, 2008. Determination of nitrofuran metabolites in milk by liquid chromatography–electrospray ionization tandem mass spectrometry. Journal of Chromatography B, 864, 156–160.

Rodziewicz L and Zawadzka I, 2008. Rapid determination of chloramphenicol residues in milk powder by liquid chromatography–elektrospray ionization tandem mass spectrometry. Talanta, 75, 846–850.

Rodziewicz L and Zawadzka I, 2013. Development and validation of a new method for determining nitrofuran metabolites in bovine urine using liquid chromatography - tandem mass spectrometry. Roczniki Panstwowego Zakladu Higieny, 64, 285–291.

Rogers GS, Belloff GB, Paul MF, Yurchenco JA and Gever G, 1956. Furazolidone, a new antimicrobial nitrofuran; a review of laboratory and clinical data. Antibiotics and Chemotherapy, 6, 231–241.

Rose GP, Dewar AJ and Stratford IJ, 1982. A biochemical neurotoxicity study relating the neurotoxic potential of metronidazole and nitrofurantoin with misonidazole. International Journal of Radiation Oncology, Biology, Physics, 8, 781–785.

Rosenberg HA and Bates TR, 1976. The influence of food on nitrofurantoin bioavailability. Clinical Pharmacology and Therapeutics, 20, 227–232.

Rosenkranz HS and Speck WT, 1976. Activation of nitrofurantoin to a mutagen by rat liver nitroreductase. Biochemical Pharmacology, 25, 1555–1556.

Rossi L, De Angelis I, Pedersen JZ, Marchese E, Stammati A, Rotilio G and Zucco F, 1996. N-[5-nitro-2-furfurylidene]-3-amino-2-oxazolidinone activation by the human intestinal cell line Caco-2 monitored through noninvasive electron spin resonance spectroscopy. Molecular Pharmacology, 49, 547–555.

Rowland IR, Mallett AK, Wise A and Bailey E, 1983. Effect of dietary carrageenan and pectin on the reduction of nitro-compounds by the rat caecal microflora. Xenobiotica, 13, 251–256.

Ryad LA, Attala E, El-Sawi SA and El-Gohary AA, 2013. Analysis of nitrofuran metabolic residues in tissues by liquid chromatography–tandem mass spectrometry. Journal of Applied Sciences Research, 9, 4567–4573.

Ryan A, Kaplan E, Laurieri N, Lowe E and Sim E, 2011. Activation of nitrofurazone by azoreductases: multiple activities in one enzyme. Scientific Reports, 1, 63.

Saari L and Peltonen K, 2004. Novel source of semicarbazide: levels of semicarbazide in cooked crayfish samples determined by LC/MS/MS. Food Additives and Contaminants, 21, 825–832.

Sachs J, Geer T, Noell P and Kunin CM, 1968. Effect of renal function on urinary recovery of orally administered nitrofurantoin. New England Journal of Medicine, 278, 1032–1035.

Sanders P, 2003. Note on analysis of nitrofurans residue semi-carbazide confirmation. Afssa (Agence Francaise de Securité Sanitaire des Aliments) and CRL (Central Reference Laboratory). Available at: http://crl.fougeres.anses.fr/publicdoc/noteCRL281103.pdf

Sapkota A, Sapkota AR, Kucharski M, Burke J, McKenzie S, Walker P and Lawrence R, 2008. Aquaculture practices and potential human health risks: current knowledge and future priorities. Environment International, 34, 1215–1226.

Sardas S, Metin A, Gok S, Karakaya AE and Aykol N, 1990. Sister chromatid exchanges in peripheral lymphocytes of urinary tract infection treated with nitrofurantoin. International Urology and Nephrology, 22, 513–517.

SCAN (Scientific Committee on Animal Nutrition), 1977. Report of the Scientific Committee on Animal Nutrition on the Use of Nitrofurans in Feedingstuffs, 9–10.

SCAN (Scientific Committee on Animal Nutrition), 1982. Report of the Scientific Committee on Animal Nutrition on the Use of Nifursol in Feedingstuffs for Turkeys, 46–50.

SCAN (Scientific Committee on Animal Nutrition), 2001. Opinion of the Scientific Committee on Animal Nutrition on the safety of use of nifursol in feedingstuffs for turkeys. Directorate C—Scientific Opinions. C2—Management of scientific committees; scientific co-operation and networks. Available at: http://ec.europa.eu/food/fs/sc/scan/out69_en.pdf

SCAN (Scientific Committee on Animal Nutrition), 2003. Update of the Opinion of the Scientific Committee on Animal Nutrition on the Safety of Product Nifursol. European Commission, Directorate General for Health and Consumer Protection. Directorate C—Scientific Opinions. C2—Management of scientific committees; science co-operation and networks.

Selby JV, Friedman GD and Fireman BH, 1989. Screening prescription drugs for possible carcinogenicity: eleven to fifteen years of follow-up. Cancer Research, 49, 5736–5747.

Setnikar I, Magistretti MJ, Veronese M, 1976. Mutagenicity studies on nifurpipone and nitrofurantoin. Proceedings of the European Society of Toxicology, 17, 405–412.

Shen Y-D, Xu Z-L, Zhang S-W, Wang H, Yang J-Y, Lei H-T, Xiao Z-L and Sun Y-M, 2012. Development of a monoclonal antibody-based competitive indirect enzyme-linked immunosorbent assay for furaltadone metabolite AMOZ in fish and shrimp samples. Journal of Agricultural and Food Chemistry, 60, 10991–10997.

Sheng L-Q, Chen M-M, Chen S-S, Du N-N, Liu Z-D, Song C-F and Qiao R, 2013. High-performance liquid chromatography with fluorescence detection for the determination of nitrofuran metabolites in pork muscle. Food Additives and Contaminants: Part A, 30, 2114–2122.

Sheu SY, Tai YT, Li WR, Lei YC, Hsieh KH, Lin CY, Cheng CC, Chang TH and Kuo TF, 2012. Development of a competitive ELISA for the detection of a furaltadone marker residue, 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ), in cultured fish samples. Journal of Veterinary Medical Science, 74, 1439–1446.

Shoda T, Yasuhara K, Moriyasu M, Takahashi T, Uneyama C, Hirose M and Mitsumori K, 2001. Testicular toxicity of nitrofurazone causing germ cell apoptosis in rats. Archives of Toxicology, 75, 297–305.

Shu J, He L, Ding H, Wang L, Guo H, Gao Y, Dzakah EE and Zeng Z, 2014. Synthesis of furaltadone metabolite, 3-amino-5-morpholinomethyl-2-oxazolidone (AMOZ) and novel haptens for the development of a sensitive enzyme-linked immunosorbent assay (ELISA). Analytical Methods, 6, 2306–2313.

Siebert D, Bayer U and Marquardt H, 1979. The application of mitotic gene conversion in *Saccharomyces cerevisiae* in a pattern of four assays, *in vitro* and *in vivo*, for mutagenicity testing. Mutation Research, 67, 145–156.

Simion G, Damiescu L and Trif A, 2012. Monitoring of the nitrofurans residues in honey bees from Timis County in the period 2007–2009. Journal of Agroalimentary Processes and Technologies, 18, 100–102.

Singh SK and Chakravarty S, 2001. Effect of nitrofurazone on the reproductive organs in adult male mice. Asian Journal of Andrology, 3, 39–44.

Slapsyte G, Jankauskiene A, Mierauskiene J and Lazutka JR, 2002. Cytogenetic analysis of peripheral blood lymphocytes of children treated with nitrofurantoin for recurrent urinary tract infection. Mutagenesis, 17, 31–35.

Slob W and Setzer RW, 2014. Shape and steepness of toxicological dose-response relationships of continuous endpoints. Critical Reviews in Toxicology, 44, 270-297.

Smith CG, Grady JE and Northam JI, 1963. Relationship between cytotoxicity *in vitro* and whole animal toxicity. Cancer Chemotherapy Reports, 30, 9–12.

Smith DJ, Paulson GD and Larsen GL, 1998. Distribution of radiocarbon after intramammary, intrauterine, or ocular treatment of lactating cows with carbon-14 nitrofurazone. Journal of Dairy Science, 81, 979–988.

Śniegocki T, Posyniak A and Żmudzki J, 2008. Determination of nitrofuran metabolite residues in eggs by liquid chromatography-mass spectrometry. Bulletin of the Veterinary Institute in Pulawy, 52, 421–425.

Song J, Yang H, Wang Y, Si W and Deng A, 2012. Direct detection of 3-amino-5-methylmorpholino-2-oxazolidinone (AMOZ) in food samples without derivatisation step by a sensitive and specific monoclonal antibody based ELISA. Food Chemistry, 135, 1330–1336.

Stadler RH, Mottier P, Guy P, Gremaud E, Varga N, Lalljie S, Whitaker R, Kintscher J, Dudler V and Read WA, 2004. Semicarbazide is a minor thermal decomposition product of azodicarbonamide used in the gaskets of certain food jars. Analyst, 129, 276–281.

Stastny K, Frgalova K, Hera A, Vass M and Franek M, 2009. In-house validation of liquid chromatography tandem mass spectrometry for determination of semicarbazide in eggs and stability of analyte in matrix. Journal of Chromatography A, 13, 8187–8191.

Steffek AJ, Verrusio AC and Watkins CA, 1972. Cleft palate in rodents after maternal treatment with various lathyrogenic agents. Teratology, 5, 33–38.

Stern IJ, Hollifield RD, Wilk S and Buzard JA, 1967. The anti-monoamine oxidase effects of furazolidone. Journal of Pharmacology and Experimental Therapeutics, 156, 492–499.

Stitzel KA, McConnell RF and Dierckman TA, 1989. Effects of nitrofurantoin on the primary and secondary reproductive organs of female B6C3F1 mice. Toxicologic Pathology, 17, 774–781.

Streeter AJ, Krueger TR and Hoener BA, 1988. Oxidative metabolites of 5-nitrofurans. Pharmacology, 36, 283–288.

Stricker BH, Blok AP, Claas FH, Van Parys GE and Desmet VJ, 1988. Hepatic injury associated with the use of nitrofurans: a clinicopathological study of 52 reported cases. Hepatology, 8, 599-606.

Swaminathan S and Lower LM Jr, 1978. Biotransformation and excretion of nitrofurans. In: Nitrofurans: chemistry, metabolism, mutagenesis and carcinogenesis. Volume 4 Carcinogenesis—A comprehensive survey. Ed. Bryan GT. Raven Press, New York, NY, USA, 59–97.

Szilagyi S and de la Calle B, 2006. Development and validation of an analytical method for the determination of semicarbazide in fresh egg and in egg powder based on the use of liquid chromatography tandem mass spectrometry. Analytica Chimica Acta, 572, 113–120.

Takahashi M, Yoshida M, Inoue K, Morikawa T and Nishikawa A, 2009. A ninety-day toxicity study of semicarbazide hydrochloride in Wistar Hannover GALAS rats. Food and Chemical Toxicology, 47, 2490–2498.

Takahashi M, Yoshida M, Inoue K, Morikawa T, Nishikawa A and Ogawa K, 2014. Chronic toxicity and carcinogenicity of semicarbazide hydrochloride in Wistar Hannover GALAS rats. Food and Chemical Toxicology, 73, 84–94.

Tang Y, Xu J, Wang W, Xiang J and Yang H, 2011a. A sensitive immunochromatographic assay using colloidal gold–antibody probe for the rapid detection of semicarbazide in meat specimens. European Food Research and Technology, 232, 9–16.

Tang Y, Xu X, Liu X, Huang X, Chen Y, Wang W and Xiang J, 2011b. Development of a Lateral Flow Immunoassay (LFA) strip for the rapid detection of 1-aminohydantoin in meat samples. Journal of Food Science, 76, T138–T143.

Tao Y, Chen D, Wei H, Yuanhu P, Liu Z, Huang L, Wang Y, Xie S and Yuan Z, 2012. Development of an accelerated solvent extraction, ultrasonic derivatisation LC-MS/MS method for the determination of the marker residues of nitrofurans in freshwater fish. Food Additives and Contaminants: Part A, 29, 736–745.

Tatsumi K, Ou T, Yoshimura H and Tsukamoto H, 1971. Metabolism of drugs. 73. The metabolic fate of nitrofuran derivatives. 1. Studies on the absorption and excretion. Chemical and Pharmaceutical Bulletin, 19, 330–334.

Tatsumi K, Nakabeppu H, Takahashi Y and Kitamura S, 1984. Metabolism *in vivo* of furazolidone: evidence for formation of an open-chain carboxylic acid and alpha-ketoglutaric acid from the nitrofuran in rats. Archives of Biochemistry and Biophysics, 234, 112–116.

Thongsrisomboon P, Liawruangrath B, Liawruangrath S and Satienperakul S, 2010. Determination of nitrofurans residues in animal feeds by flow injection chemiluminescence procedure. Food Chemistry, 123, 834–839.

Tin-Chuen Y, Sudlow G, Koch RL and Goldman P, 1983. Reduction of nitroheterocyclic compounds by mammalian tissues *in vivo*. Biochemical Pharmacology, 32, 2249–2253.

Tittlemier SA, Van de Riet J, Burns G, Potter R, Murphy C, Rourke W, Pearce H, Cao XL, Dabekai RW and Dufresne G, 2007. Analysis of veterinary drug residues in fish and shrimp composites collected during the Canadian Total Diet Study, 1993–2004. Food Additives and Contaminants, 24, 14–20.

TNO (Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek/Netherlands Organisation for Applied Scientific Research), 2004a. Bacterial reverse mutation test with semicarbazide. TNO Report no V5005/11, 6 February 2004. 22 pp. Available at: http://www.efsa.europa.eu/de/home/doc/study2_afc_doc4_en1.pdf

TNO (Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek/Netherlands Organisation for Applied Scientific Research), 2004b. Gene mutation test at the TK locus of L5178Y cells with semicarbazide. TNO Report no V5003/09, 22 January 2004. Available at: http://www.efsa.europa.eu/de/scdocs/doc/219.pdf

TNO (Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek/Netherlands Organisation for Applied Scientific Research), 2004c. Chromosomal aberration test with semicarbazide in Chinese hamster Ovary (CHO) cells. TNO Report no V 5002/09, 9 February 2004. Available at: http://www.efsa.europa.eu/de/home/doc/study1_afc_doc3_en_firstpart1.pdf

Tonomura A and Sasaki MS, 1973. Chromosome aberrations and DNA repair synthesis in cultured human cells exposed to nitrofurans. Japanese Journal of Genetics, 48, 291–294.

Toth B, Shimizu H and Erickson J, 1975. Carbamylhydrazine hydrochloride as a lung and blood vessel tumour inducer in Swiss mice. European Journal of Cancer, 11, 17–22.

Tribalat L, Paisse O, Dessalces G and Grenier-Loustalot MF, 2006. Advantages of LC-MS-MS compared to LC-MS for the determination of nitrofuran residues in honey. Analytical and Bioanalytical Chemistry, 386, 2161–2168.

Tsai C-W, Hsu C-H and Wang W-H, 2009. Determination of nitrofuran residues in tilapia tissue by enzyme-linked immunosorbent assay and confirmation by liquid chromatography tandem mass spectrometric detection. Journal of the Chinese Chemical Society, 56, 581–588.

Tsai C-W, Tang C-H and Wang W-H, 2010. Quantitative determination of four nitrofurans and corresponding metabolites in the fish muscle by liquid chromatography-electrospray ionization-tandem mass spectrometry. Journal of Food and Drug Analysis, 18, 98–106.

Uhari M, Nuutinen M and Turtinen J, 1996. Adverse reactions in children during long-term antimicrobial therapy. Pediatric Infectious Diseases Journal, 15, 404–408.

Vahl M, 2005. Analysis of nifursol residues in turkey and chicken meat using liquid chromatography-tandem mass spectrometry. Food Additives and Contaminants, 22, 120–127.

Valera-Tarifa NM, Plaza-Bolaños P, Romero-González R, Martínez-Vidal JL and Garrido-Frenich A, 2013. Determination of nitrofuran metabolites in seafood by ultra high performance liquid chromatography coupled to triple quadrupole tandem mass spectrometry. Journal of Food Composition and Analysis, 30, 86–93.

Van Poucke C, Detavernier C, Wille M, Kwakman J, Sorgeloos P and Van Peteghem C, 2011. Investigation into the possible natural occurence of semicarbazide in *Macrobrachium rosenbergii* prawns. Journal of Agricultural and Food Chemistry, 59, 2107–2112.

Vass M, Diblikova I, Cernoch I and Franek M, 2008a. ELISA for semicarbazide and its application for screening in food contamination. Analytica Chimica Acta, 608, 86–94.

Vass M, Diblikova I, Kok E, Stastny K, Frgalova K, Hruska K and Franek M, 2008b. In-house validation of an ELISA method for screening of semicarbazide in eggs. Food Additives and Contamants Part A, 25, 930–936.

Vass M, Hruska K and Franek M, 2008c. Nitrofuran antibiotics: a review on the application, prohibition and residual analysis. Veterinarni Medicina, 53, 469–500.

Verdon E, Couedor P and Sanders P, 2007. Multi-residue monitoring for the simultaneous determination of five nitrofurans (furazolidone, furaltadone, nitrofurazone, nitrofurantoin, nifursol) in poultry muscle tissue through the detection of their five major metabolites (AOZ, AMOZ, SEM, AHD, DNSAH) by liquid chromatography coupled to electrospray tandem mass spectrometry—in-house validation in line with Commission Decision 657/2002/EC. Analytica Chimica Acta, 586, 336–347.

Vinas P, Campillo N, Carrasco L and Hernandez-Cordoba M, 2007. Analysis of nitrofuran residues in animal feed using liquid chromatography and photodiode-array detection. Chromatographia, 65, 85–89.

Vlastos D, Moshou H and Epeoglou K, 2010. Evaluation of genotoxic effects of semicarbazide on cultured human lymphocytes and rat bone marrow. Food and Chemical Toxicology, 48, 209–214.

Vroomen LHM, Berghmans MCJ, Van Leeuwen P, Van Der Struijs TDB, De Vries PHU and Kuiper HA, 1986. Kinetics of 14C-furazolidone in piglets upon oral administration during 10 days and its interaction with tissue macro-molecules. Food Additives and Contaminants, 3, 331–346.

Vroomen LH, Berghmans MC, Hekman P, Hoogenboom LA and Kuiper HA, 1987a. The elimination of furazolidone and its open-chain cyano-derivative from adult swine. Xenobiotica, 17, 1427–1435.

Vroomen LH, Groten JP, van Muiswinkel K, van Velduizen A and van Bladeren PJ, 1987b. Identification of a reactive intermediate of furazolidone formed by swine liver microsomes. Chemico-Biological Interactions, 64, 167–179.

Vroomen LH, van Ommen B and van Bladeren PJ, 1987c. Quantitative studies of the metabolism of furazolidone by rat liver microsomes. Toxicology In Vitro, 1, 97–104.

Vroomen LH, Berghmans MC, Groten JP, Koeman JH and van Bladeren PJ, 1988. Reversible interaction of a reactive intermediate derived from furazolidone with glutathione and protein. Toxicology and Applied Pharmacology, 95, 53–60.

Vroomen LH, Berghmans MC, van Bladeren PJ, Groten JP, Wissink CJ and Kuiper HA, 1990. *In vivo* and *in vitro* metabolic studies of furazolidone: a risk evaluation. Drug Metabolism Review, 22, 663–676.

Wang CY and Lee LH, 1976. Mutagenic activity of carcinogenic and noncarcinogenic nitrofurans and of urine of rats fed these compounds. Chemico-Biological Interactions, 15, 69–75.

Wang CY, Croft WA and Bryan GT, 1984. Tumor production in germ-free rats fed 5-nitrofurans. Cancer Letters, 21, 303–308.

Wang J and Zhang L, 2006. Simultaneous determination and identification of furazolidone, furaltadone, nitrofurazone, and nitrovin in feeds by HPLC and LC-MS. Journal of Liquid Chromatography and Related Technologies, 29, 377–390.

Wang L, Leggas M, Goswami M, Empey PE and McNamara PJ, 2008. N-(4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]-phenyl)–9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide (GF120918) as a chemical ATP-binding cassette transporter family G member 2 (Abcg2) knockout model to study nitrofurantoin transfer into milk. Drug Metabolism and Disposition, 36, 2591–2596.

Wang X and Morris ME, 2007. Effects of the flavonoid chrysin on nitrofurantoin pharmacokinetics in rats: potential involvement of ABCG2. Drug Metabolism and Disposition, 35, 268–274.

Wang Y, Jester EL, El Said KR, Abraham A, Hooe-Rollman J and Plakas SM, 2010. Cyano metabolite as a biomarker of nitrofurazone in channel catfish. Journal of Agricultural and Food Chemistry, 58, 313–316.

Watari N, Funaki T, Aizawa K and Kaneniwa N, 1983. Nonlinear assessment of nitrofurantoin bioavailability in rabbits. Journal of Pharmacokinetics and Pharmacodynamics, 11, 529–545.

Weisburger EK, Ulland BM, Nam J, Gart JJ and Weisburger JH, 1981. Carcinogenicity tests of certain environmental and industrial chemicals. Journal of the National Cancer Institute, 67, 75–88.

Wenxiao J, Pengjie L, Xia W, Xia C, Yunfeng Z, Wei S, Xiaoping W, Yongning W and Jianzhong S, 2012. Development of an enzyme-linked immunosorbent assay for the detection of nitrofurantoin metabolite, 1-amino-hydantoin, in animal tissues. Food Control, 23, 20–25.

White AH, 1989. Absorption, distribution, metabolism, and excretion of furazolidone. A review of the literature. Scandinavian Journal of Gastroenterology, 169(Suppl.), 4–10.

Wiley MJ and Joneja MG, 1978. Neural tube lesions in the offspring of hamsters given single oral doses of lathyrogens early in gestation. Acta Anatomica, 100, 347–353.

Xia X, Li X, Zhang S, Ding S, Jiang H, Li J and Shen J, 2008. Simultaneous determination of 5-nitroimidazoles and nitrofurans in pork by high-performance liquid chromatography–tandem mass spectrometry. Journal of Chromatography A, 1208, 101–108.

Xu W, Zhu X, Wang X, Deng L and Zhang G, 2006. Residues of enrofloxacin, furazolidone and their metabolites in Nile tilapia (*Oreochromis niloticus*). Aquaculture, 254, 1–8.

Xu ZL, Zhang SW, Sun YM, Shen YD, Lei HT, Jiang YM, Eremin SA, Yang JY and Wang H, 2013. Monoclonal antibody-based fluorescence polarization immunoassay for high throughput screening of furaltadone and its metabolite AMOZ in animal feeds and tissues. Combinatorial Chemistry and High Throughput Screening, 16, 494–502.

Yahagi T, Matsushima T, Nagao M, Seino Y, Sugimura T and Bryan GT, 1976. Mutagenicities of nitrofuran derivatives on a bacterial tester strain with an R factor plasmid. Mutation Research, 40, 9–14.

Yahagi T, Nagao M, Hara K, Matsushima T, Sugimura T and Bryan GT, 1974. Relationships between the carcinogenic and mutagenic or DNA-modifying effects of nitrofuran derivatives, including 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, a food additive. Cancer Research, 34, 2266–2273.

Yamashita J, 1976. Convulsive seizure induced by intracerebral injection of semicarbazide (an anti-vitamin B6) in the mouse. Journal of Nutritional Science and Vitaminology, 22, 1–6.

Yamashita J and Hirata Y, 1977. Running fit induced by injection of semicarbazide into the superior colliculus of the mouse. Journal of Nutritional Science and Vitaminology, 23, 467–470.

Yamashita J and Hirata Y, 1978. Running fits induced by direct administration of semicarabazide into the superior colliculus of the mouse. Neuroscience Letters, 8, 89–92.

Yang W-Y, Dong J-X, Shen Y-D, Yang J-Y, Wang H, Xu Z-L, Yang X-X and Sun Y-M, 2012. Indirect competitive chemiluminescence enzyme immunoassay for furaltadone metabolite in *Metapenaeus Ensis*. Chinese Journal of Analytical Chemistry, 40, 1816–1821.

Yeung T-C and Goldman P, 1981. Inhibition of monoamine oxidase by furazolidone in germgree rats. Biochemical Pharmacology, 30, 2496–2497.

Yibar A, Centinkaya F and Soyutemiz GE, 2012. Nitrofuran metabolite 3-amino-2-oxazolidinone residues in chicken liver: a screening study. Asian Journal of Animal and Veterinary Advances, 7, 346–350.

Yibar A, Okutan B and Guezel S, 2013. Effects of boiling on nitrofuran AOZ residues in commercial eggs. Kafkas Universitesi Veteriner Fakultesi Dergisi, 19, 1023–1028.

Young RE, 1961. Intravenous furaltadone; bacteriologic, toxicologic and clinical studies. Ohio Medicine, 57, 416–417.

Yunda IF, Melnik AM and Kushniruk YI, 1974. Experimental study of the gonadotoxic effect of nitrofurans and its prevention. International Urology and Nephrology, 6, 125–135.

Zenser TV, Mattammal MB, Palmier MO and Davis BB, 1981. Microsomal nitroreductase activity of rabbit kidney and bladder: implications in 5-nitrofuran-induced toxicity. Journal of Pharmacology and Experimental Therapeutics, 219, 735–740.

Zhang Y, Wang H, Unadkat JD and Mao Q, 2007. Breast cancer resistance protein 1 limits fetal distribution of nitrofurantoin in the pregnant mouse. Drug Metabolism and Disposition, 35, 2154–2158.

Zhao Y, Zhang F and Liu Y, 2011. Determination of residues of nitrofuran metabolites in *Litopenaeus vannamei* by UPLC-MS/MS. South China Fisheries Science, 4, 008.

Zimmermann H, Gutte G, Kern M, Golbs S and Ludewig T, 1993. Basic reproductive toxicological studies of the effect of furazolidone on the hypothalamo-hypophyseo-gonadal axis of male rats. Deutsche Tierarztliche Wochenschrifte, 100, 91–94.

Zolla L and Timperio AM, 2005. Involvement of active oxygen species in protein and oligonucleotide degradation induced by nitrofurans. Biochemistry and Cell Biology, 83, 166-175.

Zuidema T, Mulder PPJ, Van Rhijn JA, Keestra NGM, Hoogenboom LA, Schat B and Kennedy DG, 2005. Metabolism and depletion of nifursol in broilers. Analytica Chimica Acta, 529, 339–346.

Zullo A, Ierardi E, Hassan C and De Francesco V, 2012. Furazolidone-based therapies for *Helicobacter pylori* infection: a pooled-data analysis. Saudi Journal of Gastroenterology, 18, 11–17.

APPENDICES

Appendix A. Sources of semicarbazide in food, other than those arising from nitruforazone use, and resulting exposures

A.1. Sources

In addition to arising from nitrofurazone use (see Section 1), SEM may occur in food from different sources, which are summarised below.

A.1.1. Azodicarbonamide use in foamed plastic gaskets

SEM is a minor thermal decomposition product of azodicarbonamide (Stadler et al., 2004), which is a blowing agent used in sealing gaskets for metal lids on glass bottles and jars (EFSA, 2005). Owing to the migration of SEM from the gasket into the food, SEM occurrence has been reported in packaged food such as baby food. The AFC Panel reported on the occurrence of SEM in baby foods and other miscellaneous foods. Data from five Member States (Finland, Germany, Ireland, Spain and the Netherlands) and industry were reported. The average concentrations in baby food were similar for the different data providers (range of mean concentrations across data providers 7–16 µg/kg) and the overall average concentration was 13 µg/kg (n = 385; concentration range 0.1–140 µg/kg). For ready-to-eat infant milk, an average concentration of 9 µg/kg was reported (n = 7; concentration range 5–14 µg/kg). For other miscellaneous foods (e.g. fruit, vegetables, jams, pickles, sauces and fish), lower levels were reported with an average concentration of 1 µg/kg (n = 121; concentration range < 0.03–10 µg/kg) (EFSA, 2005).

The use of azodicarbonamide in food contact materials has been prohibited in the EU since August 2005 (Commission Directive 2004/1/EC[39]).

A.1.2. Azodicarbonamide use as flour additive

Azodicarbonamide can be used as a flour additive to improve the physical properties of flours, particularly those low in gluten (de la Calle and Anklam, 2005). Pereira et al. (2004) estimated that the use of azodicarbonamide-treated flour for breaded chicken products can result in SEM concentrations in the breaded chicken between 0.2 and 5 µg/kg.

The use of azodicarbonamide as a flour additive is not permitted in the EU, not being included in the Community list of food additives approved for use in foods (Annex II of Regulation (EC) No 1333/2008[40]).

A.1.3. Hypochlorite treatment

High levels of SEM (up to 400 µg/kg) have been found in carrageenan[41] (Hoenicke et al., 2004). Carrageenan is prepared from red seaweed, and SEM has been detected in the raw material and was reported by the authors to occur naturally. In addition, Hoenicke et al. (2004) showed that bleaching of carrageenan with a sodium hypochlorite solution, containing 0.05–0.1 % active chlorine used for the production of processed *Euchema* seaweed (PES; a semi-refined carrageenan) results in additional formation of SEM. Based on data submitted by Marinalg, the industry association for producers of agar, alginates, carrageenan and PES, the AFC Panel reported a mean SEM concentration of 65 µg/kg in PES (n = 25; range 9–380 µg/kg (EFSA, 2005). Marinalg indicated on 11 February 2015 that there

[39] Commission Directive 2004/1/EC of 6 January 2004 amending Directive 2002/72/EC as regards the suspension of the use of azodicarbonamide as blowing agent. OJ L 7, 13.1.2004, p. 45–46.
[40] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.
[41] Carrageenan (food additive E 407) is used as a thickening, gelling and suspending agent in food, for example in ice cream, pudding, yoghurt, fruit jellies, chocolate milk and different meat products.

have been no processing changes in the production of carrageenan or PES, since the submission of the data used by the AFC Panel, that would impact the accuracy of the submitted data earlier.

SEM has also been detected in egg powder. Contamination occurred during the extraction of lysozyme from egg albumin due to the use of bleach solution to sanitise the carrageenan column used for the extraction of lysozyme. However, another possible cause of SEM in egg powder could be the heat treatment (EFSA, 2005).

Hoenicke et al. (2004) studied the formation of SEM in foods treated with hypochlorite solution. SEM was formed in chicken, egg white powder, carrageenan, locust bean gum, gelatine and starch, after overnight treatment with a hypochlorite solution containing 1 % active chlorine. The increase in SEM formation following hypochlorite treatment was in the range of the method variability for shrimps, milk, soybean flakes and red seaweed. Treatment with a hypochlorite solution containing 0.015 % active chlorine resulted in only a little formation of SEM in carrageenan and starch.

The AFC Panel evaluated the occurrence of SEM in food due to the use of chlorinated water as a processing aid.[42] Given that the concentrations used by the food industry are 100- to 1 000-fold lower than the concentration of 0.015 % active chlorine that gave barely any detectable SEM formation in the tests performed by Hoenicke et al. (2004), given that the chlorine wash will be for a far shorter period than the overnight conditions used in the laboratory tests, and given that the processes also generally incorporate a final rinse with chilled water with just 0.0002 to 0.0004 % free chlorine, the AFC Panel concluded that the use of chlorinated water as a processing aid is highly unlikely to give any detectable residues of SEM in the washed food.

The AFC Panel also considered disinfection of equipment and surfaces with disinfecting agents such as sodium hypochlorite and concluded that, with effective rinsing, no subsequent formation of SEM is to be expected (EFSA, 2005).

A.1.4. Natural occurrence

Natural occurrence of SEM has been reported in shrimps/prawns, seaweed, crayfish and honey, mostly at a concentration below the MRPL of 1 µg/kg, but concentrations up to 12 µg/kg in crayfish have been reported (Hoenicke et al., 2004; Saari and Peltonen, 2004; Van Poucke et al., 2011; Crews, 2014; McCracken et al., 2013)

A.2. Exposure

In 2005, the AFC Panel estimated the exposure to the different sources of SEM described above (EFSA, 2005).

For a 9-month old infant of 8.8 kg b.w. eating exclusively food and drink from glass jars and bottles containing SEM, the AFC Panel estimated that the exposure to SEM would be 0.35 µg/kg b.w. per day for an average consumer (of the category 'consumers only') and 0.69 µg/kg b.w. per day for a high consumer (95th percentile). For an infant (4.5 kg b.w.) consuming only pre-packaged infant milk in glass bottles with metal lids, the intake was estimated to be 1.4 µg/kg b.w. per day. For adults, the exposure is considerably lower. Assuming the consumption of 1 kg of food contaminated with SEM at an average concentration of 1 µg/kg, the exposure for an adult (60 kg b.w.) would be 0.02 µg/kg b.w. per day. However, given that the use of azodicarbonamide in food contact materials is prohibited in the EU, the European population should no longer be exposed via this route.

[42] Chlorinated water may be used as a processing aid to wash foods, e.g. fruit and vegetables, provided that it meets the definition of a processing aid, i.e. it does not perform a function such as preservation in the final product and leaves no harmful residues (Directive 89/107/EEC). It is a reasonably widespread practice to wash certain ready-to-eat foods using water with a chlorine content up to 0.0001 % (EFSA, 2005).

The dietary exposure from breaded meat products imported into the EU was estimated to be 1 µg/person considering the consumption of 200 g of product containing 5 µg/kg SEM. This exposure corresponds to 17 ng/kg b.w. per day for a 60 kg b.w. person. No dietary exposure from imported bread and bakery ware was estimated, as the import of these products into the EU is probably very low.

For a high consumer of egg products, the exposure to SEM was estimated to be 8 ng/kg b.w. per day for a 60 kg b.w. person.

The exposure could be up to 5 ng/kg b.w. per day from the use of carrageenan, assuming that consumption was up to the full ADI for carrageenan (75 mg/kg b.w. per day) and that all consumed carrageenan contained SEM at 65 µg/kg.

In addition, the AFC Panel noted that SEM may be formed at low levels during drying of some foods, it may be present at very low background levels naturally or it may also derive from as yet unidentified sources.

Appendix B. Occurrence data

Table B.1: Number of samples analysed for the nitrofuran marker metabolites (3-amino-2-oxazolidinone, 3-amino-5-methylmorpholino-2-oxazolidinone, 1-aminohydantoin, semicarbazide) present in the EFSA Chemical Occurrence database

Country	EFSA Foodex level 2	Year of sampling			
		2010	2011	2012	2013
Czech Republic[(a)]	Eggs, fresh			40	4
	Fish meat			36	
	Livestock meat			252	16
	Poultry			148	16
	Game mammals				4
	Liquid milk			48	
	Honey			16	4
Denmark[(b)]	Composite food (including frozen products)	4			
	Eggs, fresh	152	160	160	200
	Fish meat	68	28	116	68
	Crustaceans	184	260	276	816
	Fish and other seafood (including amphibians, reptiles, snails and insects)		104		
	Livestock meat	1 288	1 300	1 308	1 340
	Poultry	320	284	340	284
	Edible offal, farmed animals	460	312	256	
	Honey		52	20	72
Spain[(c)]	Ready-to-eat meals for infants and young children			40	

(a): All results for samples reported by the Czech Republic are below the reported LODs (LODs ≤ 1 µg/kg).
(b): All results for samples reported by Denmark are below the reported CCβ values (CCβ values ≤ 1 µg/kg).
(c): All results for samples reported by Spain are below the reported CCα values (CCα values ≤ 1 µg/kg).

Appendix C. Consumption data

Table C.1: Dietary surveys considered for the chronic dietary exposure assessment with the number of subjects in the different age classes

Code[a]	Country	Dietary survey[b]	Method	Days	Age (years)	Number of subjects[c]						
						Infants	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
BE/1	Belgium	Diet National 2004	24-hour dietary recall	2	15–105				584	1 304	518	712
BE/2	Belgium	Regional Flanders	Food record	3	2–5		36[d]	625				
BG/1	Bulgaria	NUTRICHILD	24-hour recall	2	0.1–5	860	428	433				
CZ	Czech Republic	SISP04	24-hour recall	2	4–64			389	298	1 666		
DK	Denmark	Danish Dietary Survey	Food record	7	4–75			490	479	2 822	309	20[d]
DE/1	Germany	DONALD 2006–2008	Dietary record	3	1–10		261	660				
DE/2	Germany	National Nutrition Survey II	24-hour recall	2	14–80				1 011	10 419	2 006	490
IE	Ireland	NSFC	Food record	7	18–64					958		
EL	Greece	Regional Crete	Dietary record	3	4–6			839				
ES/1	Spain	AESAN	Food record	3	18–60					410		
ES/2	Spain	AESAN-FIAB	24-hour recall	2	17–60				86	981		
ES/3	Spain	NUT INK05	24-hour recall	2	4–18			399	651			
ES/4	Spain	enKid	24-hour recall	2	1–14		17[d]	156	209			
FR	France	INCA2	Food record	7	3–79			482	973	2 276	264	84
IT	Italy	INRAN-SCAI 2005–06	Food record	3	0.1–98	16[d]	36[d]	193	247	2 313	290	228
CY	Cyprus	Childhealth	Dietary record	3	11–18				303			
LV	Latvia	EFSA_TEST	24-hour recall	2	7–66			189	470	1 306		
HU	Hungary	National Repr Surv	Food record	3	18–96					1 074	206	80
NL/1	Netherlands	DNFCS 2003	24-hour dietary recall	2	19–30					750		
NL/2	Netherlands	VCP kids	Food record	3	2–6		322	957				
FI/1	Finland	DIPP	Food record	3	1–6		497	933				
FI/2	Finland	FINDIET 2007	48-hour recall	2	25–74					1 575	463	
FI/3	Finland	STRIP	Food record	4	7–8			250				
SE/1	Sweden	RIKSMATEN 1997–98	Food record	7	18–74					1 210		
SE/2	Sweden	NFAn	24-hour recall	4	3–18			1 473	1 018			

Code[(a)]	Country	Dietary survey[(b)]	Method	Days	Age (years)	Number of subjects[(c)]						
						Infants	Toddlers	Other children	Adolescents	Adults	Elderly	Very elderly
UK	United Kingdom	NDNS	Food record	7	19–64					1 724		

(a): Abbreviations to be used consistently in all tables on exposure assessment.
(b): More information on the dietary surveys is given in the EFSA guidance 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011b).
(c): Number of available subjects for chronic exposure assessment in each age class.
(d): 95th percentiles calculated over a number of observations fewer than 60. These require cautious interpretation, as the results may not be statistically robust (EFSA, 2011b).

Appendix D. Dietary exposure for scenario 1B

Table D.1: Summary statistics for the hypothetical chronic dietary exposure (ng/kg b.w. per day) to nitrofuran marker metabolites estimated by age class for scenario 1B.

Age class	Number of surveys	Scenario 1B[(a)]		
		Minimum	Median	Maximum
Mean dietary exposure				
Infants	2	20.2	–[(b)]	82
Toddlers	7	24.6	36	44
Other children	15	9.5	23	39
Adolescents	12	4.8	9.4	14
Adults	15	4.4	6.8	10
Elderly	7	4.1	5.1	7.8
Very elderly	6	4.1	5.3	7.8
95th percentile dietary exposure[(c)]				
Infants	1	–[(d)]	–[(d)]	–[(d)]
Toddlers	4	53	57	103
Other children	15	18	40	67
Adolescents	12	11	18	27
Adults	15	9.5	13	17
Elderly	7	8.3	9.4	16
Very elderly	5	8.3	9.5	11

The minimum, median and maximum of the mean and 95th percentile exposure values across dietary surveys in European countries are shown.
In order to avoid the impression of too high precision, the numbers for all exposure estimates are rounded to two figures.
b.w.: body weight; RPA: reference point for action.
(a): Scenario 1B contains foods of animal origin, including milk and dairy products, that are contaminated with one nitrofuran marker metabolite at a concentration equal to the RPA value of 1 µg/kg.
(b): Not calculated; estimates available from only two dietary surveys.
(c): The 95th percentile estimates obtained from dietary surveys/age classes with fewer than 60 observations may not be statistically robust (EFSA, 2011b) and therefore are not included in this table.
(d): Estimates available from only one dietary survey: 73 ng/kg b.w. per day.

Appendix E. Semicarbazide

Table E.1: Concentration of semicarbazide (SEM) in the final product as calculated for the different food categories of non-animal products and milk and dairy products, for which carrageenan is authorised as an additive and their equivalent FoodEx1 category, based on the maximum usage levels reported to EFSA.[43] Usage levels were reported to EFSA through a public call for data; when a usage level was not reported, the concentration of 1 µg/kg was considered.

Food category authorisation	MPL	Food group[(a)]	FoodEx1 code	FoodEx1 description	Maximum usage level of carrageenan in the final product (mg/kg)	Concentration of SEM in the final product (µg/kg)
Unflavoured fermented milk products, heat-treated after fermentation (1.3) (legislation: (EU) No 1129/2011, applicable as from 01/06/2013)	qs	1.3	A.01.001028	Yoghurt, cow milk, plain	10 000	0.65
			A.01.001036	Yoghurt, sheep milk		
			A.01.001037	Yoghurt, goat milk		
			A.01.001038	Sour milk		
			A.01.001039	Acidophilus milk		
			A.01.001040	Kefir		
			A.01.001041	Buttermilk		
			A.01.001042	Kumis		
			A.01.001043	Cieddu		
			A.01.001044	Kaeder milk		
			A.01.001045	Skyr		
			A.01.001046	Taette		
			A.01.001047	Filmjölk		
			A.01.001048	Viili		
Flavoured fermented milk products including heat-treated products (1.4) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	1.4	A.01.001032	Yoghurt, cow milk, with fruit	5 000	0.33
Dehydrated milk as defined by Directive 2001/114/EC (1.5) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	1.5	A.01.000981	Dried milk	nr	1.00

Table continued overleaf.

[43] Call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Published: 27 March 2013. Deadline 15 September 2013. Available at: http://www.efsa.europa.eu/en/data/call/130327.htm

Table E.1: Concentration of semicarbazide (SEM) in the final product as calculated for the different food categories of non-animal products and milk and dairy products, for which carrageenan is authorised as an additive and their equivalent FoodEx1 category, based on the maximum usage levels reported to EFSA. Usage levels were reported to EFSA through a public call for data; when a usage level was not reported, the concentration of 1 µg/kg was considered. (continued)

Food category authorisation	MPL	Food group[(a)]	FoodEx1 code	FoodEx1 description	Maximum usage level of carrageenan in the final product (mg/kg)	Concentration of SEM in the final product (µg/kg)
Unflavoured pasteurised cream (excluding reduced fat creams) (1.6.1) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	1.6.1	A.01.001000	Cream and cream products	5 500	0.36
Unflavoured live fermented cream products and substitute products with a fat content of less than 20 % (1.6.2)	qs	1.6.2	Same as for unflavoured live fermented cream products and substitute products with a fat content of less than 20 % (1.6.2)		5 500	0.36
Other creams (1.6.3) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	1.6.3	Same as for unflavoured live fermented cream products and substitute products with a fat content of less than 20 % (1.6.2)		5 500	0.36
Unripened cheese excluding products falling in category 16 (1.7.1), except mozzarella	qs	1.7.1	A.01.001054 A.01.001055	Quark Quark with fruit	6 300	0.41
Processed cheese (1.7.5) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	1.7.5	A.01.001056 A.01.001057 A.01.001058 A.01.001059 A.01.001060 A.01.001061	Cheese, processed, sliceable Cheese, processed spreadable Cheese, processed, with condiments Cheese, processed, with ham Cheese, processed, with mushrooms Cheese, processed, with pepper herbs	5 000	0.33

Table continued overleaf.

Table E.1: Concentration of semicarbazide (SEM) in the final product as calculated for the different food categories of non-animal products and milk and dairy products, for which carrageenan is authorised as an additive and their equivalent FoodEx1 category, based on the maximum usage levels reported to EFSA. Usage levels were reported to EFSA through a public call for data; when a usage level was not reported, the concentration of 1 µg/kg was considered. (continued)

Food category authorisation	MPL	Food group[(a)]	FoodEx1 code	FoodEx1 description	Maximum usage level of carrageenan in the final product (mg/kg)	Concentration of SEM in the final product (µg/kg)
Processed cheese (1.7.5) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	1.7.5	A.01.001063 A.01.001064	Cheese, processed, low fat Cheese, processed cheese, plain		
Cheese products (excluding products falling in category 16) (1.7.6) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	1.7.6	Same as for processed cheese (1.7.5) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)		5 000	0.33
Dairy analogues, including beverage whiteners (1.8) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	1.8	A.01.001240	Milk and milk product imitates	117	0.08
Other fat and oil emulsions including spreads, as defined by Council Regulation (EC) No 1234/2007, and liquid emulsions (2.2.2) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	2.2.2	A.01.001389	Margarine and similar products	nr	1.00
Vegetable oil pan spray (2.3) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	2.3	A.01.001362	Vegetable fat	nr	1.00
Edible ices (3) (legislation: (EU) No 1129/2011, applicable as from 01/06/2013)	qs	3	A.01.001888	Ices and desserts	10 000	0.65
Dried fruit and vegetables (4.2.1)	qs	4.2.1	A.01.000647 A.01.000683	Dried fruits Mixed dried fruits	10 000 10 000	0.65 0.65
Fruit and vegetables in vinegar, oil, or brine (4.2.2) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	4.2.2	A.01.000723	Fruit in vinegar, oil, or brine	10 000	0.65

Table continued overleaf.

Table E.1: Concentration of semicarbazide (SEM) in the final product as calculated for the different food categories of non-animal products and milk and dairy products, for which carrageenan is authorised as an additive and their equivalent FoodEx1 category, based on the maximum usage levels reported to EFSA. Usage levels were reported to EFSA through a public call for data; when a usage level was not reported, the concentration of 1 µg/kg was considered. (continued)

Food category authorisation	MPL	Food group[(a)]	FoodEx1 code	FoodEx1 description	Maximum usage level of carrageenan in the final product (mg/kg)	Concentration of SEM in the final product (µg/kg)
Fruit and vegetable preparations excluding compote (4.2.4.1) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	4.2.4.1	A.01.000684	Fruit salad	10 000	0.65
			A.01.000685	Fruit chips		
			A.01.000686	Fruit, purée		
			A.01.000687	Fruit cocktail		
			A.01.000714	Candied fruits		
			A.01.000724	Fermented fruit products		
			A.01.000725	Fruit fillings for pastries		
			A.01.000726	Fruit, chocolate coated		
			A.01.000449	Coconut milk (*Cocos nucifera*)	300	0.02
Jam, jellies and marmalades and sweetened chestnut purée, as defined by Directive 2001/113/EC (4.2.5.2) (legislation: (EC) No 1333/2008, applicable as of 16/12/2008)	10 000	4.2.5.2	A.01.000657	Jam, marmalade and other fruit spreads	10 000	0.65
Other similar fruit or vegetable spreads (4.2.5.3) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	10 000	4.2.5.3	Covered under jam, marmalade and other fruit spreads		10 000	0.65
Nut butters and nut spreads (4.2.5.4) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	4.2.5.4	Covered under vegetable fats			
Processed potato products (4.2.6) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	4.2.6	A.01.000471	French fries	8 000	0.52
			A.01.000477	Potato croquettes		
			A.01.001879	Potato crisps		

Table continued overleaf.

Table E.1: Concentration of semicarbazide (SEM) in the final product as calculated for the different food categories of non-animal products and milk and dairy products, for which carrageenan is authorised as an additive and their equivalent FoodEx1 category, based on the maximum usage levels reported to EFSA. Usage levels were reported to EFSA through a public call for data; when a usage level was not reported, the concentration of 1 µg/kg was considered. (continued)

Food category authorisation	MPL	Food group[(a)]	FoodEx1 code	FoodEx1 description	Maximum usage level of carrageenan in the final product (mg/kg)	Concentration of SEM in the final product (µg/kg)
Cocoa and chocolate products, as covered by Directive 2000/36/EC (5.1) ,only energy-reduced or with no added sugars	qs	5.1	A.01.001295	Chocolate (Cocoa) products	1 913	0.12
			A.01.000421	Cocoa beverage-preparation, powder	18 000	1.17
			A.01.001532	Hot chocolate	288	0.02
Other confectionery including breath refreshening microsweets (5.2) not be used in jelly mini-cups, defined, for the purpose of this Regulation, as jelly confectionery of a firm consistency, contained in semi rigid mini-cups or mini-capsules, intended to be ingested in a single bite by exerting pressure on the mini-cups or mini-capsule to project the confectionery into the mouth	qs	5.2	A.01.001311	Candies, with sugar	980	0.06
			A.01.001312	Candies, sugar free		
			A.01.001314	Caramel, hard		
			A.01.001315	Caramel, soft		
			A.01.001316	Toffee		
			A.01.001317	Fudge		
			A.01.001318	Dragée, sugar coated		
			A.01.001321	Liquorice candies		
			A.01.001322	Gum drops		
			A.01.001323	Jelly candies		
Chewing gum (5.3) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	5.3	A.01.001326	Chewing gum with added sugar	15 000	0.98
			A.01.001327	Chewing gum without added sugar		
Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4 (5.4) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	5.4	A.01.001310	Confectionery (non-chocolate)	6 500	0.42
Starches (6.2.2) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	6.2.2	Not considered, as the codes covering starches in the FoodEx1 classification are milling products and not part of recipes			

Table continued overleaf.

Table E.1: Concentration of semicarbazide (SEM) in the final product as calculated for the different food categories of non-animal products and milk and dairy products, for which carrageenan is authorised as an additive and their equivalent FoodEx1 category, based on the maximum usage levels reported to EFSA. Usage levels were reported to EFSA through a public call for data; when a usage level was not reported, the concentration of 1 µg/kg was considered. (continued)

Food category authorisation	MPL	Food group[(a)]	FoodEx1 code	FoodEx1 description	Maximum usage level of carrageenan in the final product (mg/kg)	Concentration of SEM in the final product (µg/kg)
Breakfast cereals (6.3) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	6.3	A.01.000184	Breakfast cereals	3 900	0.25
Dry pasta (6.4.2), only gluten free and/or pasta intended for hypoproteic diets	qs	6.4.2	A.01.000183	Pasta, gluten free	nr	1.00
Potato gnocchi (6.4.4) except fresh refrigerated potato gnocchi	qs	6.4.4	Not specifically specified under the FoodEx1 classification system			
Fillings of stuffed pasta (ravioli and similar) (6.4.5) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	6.4.5	Not specifically specified under the FoodEx1 classification system			
Noodles (6.5) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	6.5	Not considered, as it is not specifically specified under the FoodEx1 classification system			
Batters (6.6) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	6.6	Covered under fine bakery wares			
Pre-cooked or processed cereals (6.7) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	6.7	Covered under the breakfast cereal			
Bread and rolls (7.1), except products in 7.1.1 and 7.1.2	qs	7.1	A.01.000098	Bread and rolls	3 900	0.25
Fine bakery wares (7.2) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	7.2	A.01.000252 A.01.000253 A.01.000302	Fine bakery wares Pastries and cakes Biscuits (cookies)	3 900 20 000 3 291	0.25 1.30 0.21
Other sugars and syrups (11.2) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	11.2	A.01.001333	Molasses and other syrups	12 000	0.78
Table-top sweeteners in liquid form (11.4.1) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs[3]	11.4.1	A.01.001280	Sugar substitutes	nr	1.00

Table continued overleaf.

Table E.1: Concentration of semicarbazide (SEM) in the final product as calculated for the different food categories of non-animal products and milk and dairy products, for which carrageenan is authorised as an additive and their equivalent FoodEx1 category, based on the maximum usage levels reported to EFSA. Usage levels were reported to EFSA through a public call for data; when a usage level was not reported, the concentration of 1 µg/kg was considered. (continued)

Food category authorisation	MPL	Food group[(a)]	FoodEx1 code	FoodEx1 description	Maximum usage level of carrageenan in the final product (mg/kg)	Concentration of SEM in the final product (µg/kg)
Table-top sweeteners in powder form (11.4.2) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs[3]	11.4.2	Covered by sugar substitutes			
Salt substitutes (12.1.2) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	12.1.2	Not specifically specified under the FoodEx1 classification system			
Seasonings and condiments (12.2.2) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	12.2.2	A.01.001649	Condiment	3 300	0.21
Vinegars (12.3) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	12.3	Covered by condiments			
Mustard (12.4) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	12.4	Covered by condiments			
Soups and broths (12.5) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	12.5	A.01.001856	Ready-to-eat soups	109	0.01
Sauces (12.6) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	12.6	A.01.001684	Savoury sauces	9 900	0.64
Salads and savoury-based sandwich spreads (12.7) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	12.7	A.01.001665	Dressing	5 000	0.33
Yeast and yeast products (12.8) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	12.8	Not specifically specified under the FoodEx1 classification system			
Protein products, excluding products covered in category 1.8 (12.9) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	12.9	Not specifically specified under the FoodEx1 classification system			
Follow-on formulae, as defined by Directive 2006/141/EC (13.1.2)	300	13.1.2	A.01.001722	Follow-on formulae	300	0.00

Table continued overleaf.

Table E.1: Concentration of semicarbazide (SEM) in the final product as calculated for the different food categories of non-animal products and milk and dairy products, for which carrageenan is authorised as an additive and their equivalent FoodEx1 category, based on the maximum usage levels reported to EFSA. Usage levels were reported to EFSA through a public call for data; when a usage level was not reported, the concentration of 1 µg/kg was considered. (continued)

Food category authorisation	MPL	Food group[(a)]	FoodEx1 code	FoodEx1 description	Maximum usage level of carrageenan in the final product (mg/kg)	Concentration of SEM in the final product (µg/kg)
Other foods for young children (13.1.4)	300	13.1.4	A.01.001728	Cereal-based food for infants and young children	300	0.00
			A.01.001733	Ready-to-eat meals for infants and young children		
			A.01.001739	Yoghurt, cheese and milk-based desserts for infants and young children		
			A.01.001743	Fruit juice and herbal tea for infants and young children		
Dietary foods for special medical purposes, as defined in Directive 1999/21/EC (excluding products from food category (13.1.5) (13.2) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	13.2	A.01.001784	Medical food (are specially formulated and intended for the dietary management of a disease that has distinctive nutritional needs that cannot be met by normal diet alone; intended to be used under medical supervision)	8 000	0.52
Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet) (13.3) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	13.3	A.01.001749	Food for weight reduction	5 000	0.33

Table continued overleaf.

Table E.1: Concentration of semicarbazide (SEM) in the final product as calculated for the different food categories of non-animal products and milk and dairy products, for which carrageenan is authorised as an additive and their equivalent FoodEx1 category, based on the maximum usage levels reported to EFSA. Usage levels were reported to EFSA through a public call for data; when a usage level was not reported, the concentration of 1 µg/kg was considered. (continued)

Food category authorisation	MPL	Food group[(a)]	FoodEx1 code	FoodEx1 description	Maximum usage level of carrageenan in the final product (mg/kg)	Concentration of SEM in the final product (µg/kg)
Foods suitable for people intolerant to gluten, as defined by Regulation (EC) No 41/2009 (13.4), including dry pasta	qs	13.4	Covered under bread and rolls			
Fruit juices, as defined by Directive 2001/112/EC, and vegetable juices (14.1.2), only vegetables juices	qs	14.1.2	A.01.001394	Fruit and vegetable juices	450	0.03
Fruit nectars, as defined by Directive 2001/112/EC, and vegetable nectars and similar products (14.1.3), only vegetable nectars	qs	14.1.3	Covered under fruit and vegetable juices			
Flavoured drinks (14.1.4) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	14.1.4	A.01.001471	Soft drinks	450	0.03
Other (14.1.5.2), excluding unflavoured leaf tea, including flavoured instant coffee	qs	14.1.5.2	A.01.000963 A.01.001527	Milk-based beverages Iced coffee	500 250	0.03 0.02
Cider and perry (14.2.3) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	14.2.3	A.01.001549	Wine-like drinks (e.g. cider, perry)	20	0.00
Fruit wine and made wine (14.2.4) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	14.2.4	Covered by wine-like drinks		20	0.00
Mead (14.2.5) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	14.2.5	Covered under wine-like drinks		20	0.00
Spirit drinks, as defined in Regulation (EC) No 110/2008 (14.2.6) ,except whisky or whiskey	qs	14.2.6	A.01.001561	Spirits	20	0.00
Aromatised wines (14.2.7.1) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	14.2.7.1	Covered under wine-like drinks		20	0.00

Table continued overleaf.

Table E.1: Concentration of semicarbazide (SEM) in the final product as calculated for the different food categories of non-animal products and milk and dairy products, for which carrageenan is authorised as an additive and their equivalent FoodEx1 category, based on the maximum usage levels reported to EFSA. Usage levels were reported to EFSA through a public call for data; when a usage level was not reported, the concentration of 1 µg/kg was considered. (continued)

Food category authorisation	MPL	Food group[(a)]	FoodEx1 code	FoodEx1 description	Maximum usage level of carrageenan in the final product (mg/kg)	Concentration of SEM in the final product (µg/kg)
Aromatised wine-based drinks (14.2.7.2) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	14.2.7.2	Covered under wine-like drinks		20	0.00
Aromatised wine-product cocktails (14.2.7.3) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	14.2.7.3	A.01.001569	Alcoholic mixed drinks	20	0.00
Other alcoholic drinks, including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of alcohol (14.2.8)	qs	14.2.8	Covered under alcoholic mixed drinks			
Potato-, cereal-, flour- or starch-based snacks (15.1) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	15.1	A.01.001878	Snack food	8 000	0.52
Processed nuts (15.2) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	15.2	Covered under snack food			
Desserts, excluding products covered in categories 1, 3 and 4 (16) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	16	Covered under ices and desserts		10 000	0.65
Food supplements supplied in a solid form, including capsules and tablets and similar forms, excluding chewable forms (17.1)	qs	17.1	A.01.001752	Dietary supplements	50 488	3.28
Food supplements supplied in a liquid form (17.2) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	17.2	Covered under dietary supplements		50 488	3.28

Table continued overleaf.

Table E.1: Concentration of semicarbazide (SEM) in the final product as calculated for the different food categories of non-animal products and milk and dairy products, for which carrageenan is authorised as an additive and their equivalent FoodEx1 category, based on the maximum usage levels reported to EFSA. Usage levels were reported to EFSA through a public call for data; when a usage level was not reported, the concentration of 1 µg/kg was considered. (continued)

Food category authorisation	MPL	Food group[(a)]	FoodEx1 code	FoodEx1 description	Maximum usage level of carrageenan in the final product (mg/kg)	Concentration of SEM in the final product (µg/kg)
Food supplements supplied in a syrup-type or chewable form (17.3) (legislation: (EU) No 1129/2011, applicable as of 01/06/2013)	qs	17.3	Covered under dietary supplements		50 488	3.28
Processed foods not covered by categories 1 to 17, excluding foods for infants and young children (18) (legislation: (EU) No 1333/2008, applicable as of 16/12/2008)	qs	18	Covered under other food categories			

MPL: maximum permitted level; nr: not reported to EFSA; qs: *quantum satis*.
(a): Food group is as defined in Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives. OJ L 354, 31.12.2008, p. 16.

Appendix F. Dietary exposure for scenarios 2B and 2D

Table F.1: Summary statistics for the hypothetical chronic dietary exposure (ng/kg b.w. per day) to nitrofuran marker metabolites estimated by age class for scenarios 2B and 2D.

Age class	Number of surveys	Scenario 2B[a]			Scenario 2D[b]		
		Minimum	Median	Maximum	Minimum	Median	Maximum
Mean dietary exposure							
Infants	2	10	–[c]	11	7.7	–[c]	12
Toddlers	7	10	21	47	7.7	13	21
Other children	15	16	24	35	8.5	11	18
Adolescents	12	11	14	18	4.4	6.8	8.2
Adults	15	6.0	8.9	15	3.7	5.0	6.3
Elderly	7	4.4	6.7	9.1	3.4	4.0	4.9
Very elderly	6	5.2	6.5	9.2	3.2	4.3	4.7
95th percentile dietary exposure[d]							
Infants	1	–[e]	–[e]	–[e]	–[f]	–[f]	–[f]
Toddlers	4	35	42	64	15	25	39
Other children	15	32	42	68	14	19	32
Adolescents	12	19	26	35	8.2	12	16
Adults	15	11	17	28	6.5	8.7	10.2
Elderly	7	8.8	11	17	5.6	7.1	8.3
Very elderly	5	8.5	11	17	5.4	8.0	8.4

The minimum, median and maximum of the mean and 95th percentile exposure values across dietary surveys in European countries are shown
To avoid the impression of too high precision, the numbers for all exposure estimates are rounded to two figures.
b.w.: body weight; SEM: semicarbazide; RPA: reference point for action.

(a): Scenario 2B contains foods of animal origin, excluding milk and dairy products, and foods of non-animal origin, for which carrageenan is authorised as an additive, contaminated with SEM at a concentration equal to the RPA level of 1 µg/kg.

(b): Scenario 2D contains foods of animal origin, excluding milk and dairy products, contaminated with SEM at a concentration equal to the RPA level of 1 µg/kg, and foods of non-animal origin and milk and dairy products, for which carrageenan is authorised as an additive, contaminated with SEM at concentrations calculated from maximum usage levels of carrageenan and actual concentrations of SEM in carrageenan.

(c): Not calculated; estimates available only from two dietary surveys.

(d): The 95th percentile estimates obtained from dietary surveys/age classes with fewer than 60 observations may not be statistically robust (EFSA, 2011b) and therefore were not included in this table.

(e): Estimates available from only one dietary survey: 29 ng/kg b.w. per day.

(f): Estimates available from only one dietary survey: 48 ng/kg b.w. per day.

Appendix G. Acute toxicity

Table G.1: Medium lethal doses (LD_{50}) for furazolidone, 3-amino-2-oxazolidinone (AOZ), 3-amino-5-methylmorpholino-2-oxazolidinone (AMOZ), nitrofurantoin, nitrofurazone and semicarbazide (SEM)

Animal species (age)	Route of administration	LD_{50} (mg/kg b.w.)	Comment	Reference
Furazolidone				
Rat (adult)	Oral	1 508	Yellow powder in mixture with 1 % methylcellulose	Mitchell et al. (1990a)
Mouse (adult)	Oral	1 110	Yellow powder in mixture with 1 % methylcellulose	Mitchell et al. (1990b)
AOZ				
Rat	Oral	2 739	Colourless crystals in distilled water	NOTOX (1994b)
AMOZ				
Rat	Oral	> 2 000	Colourless crystals	NOTOX (1994a)
Nitrofurantoin				
Rat (60 days)	Oral	1 493	Nitrofurantoin sodium	Goldenthal (1971)
Rat (1 day)	Oral	89	Nitrofurantoin sodium	Goldenthal (1971)
Rat (male)	Oral	604	5–15 % acacia in water	Preti (1970), as cited by NTP (1989)
Rat (male)	i.p.	112	5–15 % acacia in water	Preti (1970), as cited by NTP (1989)
Rat (50 days)	s.c.	178	Nitrofurantoin sodium	Goldenthal (1971
Rat (1 day)	s.c.	152	Nitrofurantoin sodium	Goldenthal (1971
Rat (adult)	s.c.	35	Macrocrystals, vitamin E deficient diet	Boyd et al. (1979)
Rat (adult)	s.c.	400	Macrocrystals	Boyd et al. (1979)
Mice	oral	360	Chemical form not specified	NIOSH (1983), as cited by NTP (1989)
Mice	i.p.	150	Chemical form not specified	NIOSH (1983), as cited by NTP (1989)
Chicken	Oral	148	Macrocrystals	Peterson et al. (1982)
Chicken	Oral	53	Macrocrystals, selenium and vitamin E deficient diet	Peterson et al. (1982)
Nitrofurazone				
Rat	Oral	590	Crystals in acacia solution	Krantz and Evans (1945)
Rat	Oral	590	Crystals	Miyaji (1971)
Rat	Oral	800	Chemical form not specified	Anderson (1983), as cited by FAO/WHO, (1989b)
Mice	Oral	380	Macrocrystals	Krantz and Evans (1945)
Mice	Oral	640	Macrocrystals	Miyaji (1971)

Animal species (age)	Route of administration	LD_{50} (mg/kg b.w.)	Comment	Reference
Mice	Oral	587[(a)] (460 to 582)[(b)]	Needle-like solid in gum acacia (10 %)	Dodd (1946)
Mice	i.p.	300	Chemical form not specified	Smith et al. (1963)
SEM				
Rats	i.p.	212	Chemical form not specified	De la Fuente del Rey (1986)
Mice	i.p.	123.3	Chemical form not specified	Jenney et al. (1958)
Mice	s.c.	125.5	Chemical form not specified	Jenney et al. (1958)
Mice	i.v.	125.6	Chemical form not specified	Jenney et al. (1958)
Mice	Oral	176	Chemical form not specified	Jenney et al. (1958)

b.w.: body weight; LD_{50}: median lethal dose.
(a): LD_{50} when a mixture of seven lots was administered.
(b): Range of LD_{50}s for seven different lots.

Appendix H. *In vitro* and *in vivo* genotoxicity studies

Table H.1: *In vitro* and *in vivo* genotoxicity studies of furazolidone

Test organism/system	Method	Concentration/ Treatment	Metabolic activation	Outcome	Reference
Vibrio cholera	Forward mutation assay	Up to 12 µg/mL	– S9 mix	Dose-dependent streptomycin resistance forward mutation	Chatterjee et al. (1983)
Escherichia coli PQ37	SOS Chromotest	0.05–25 nM/mL	– S9 mix	Dose-dependent induction of SOS response	Akinshina et al. (1992)
Salmonella enterica subsp. *enterica* serovar Typhimurium TA1535/pSK1002	SOS/umu assay	Up to 12 µg/mL	– S9 mix	Dose-dependent increase in the SOS response	Pal et al. (1992)
E coli WP2; WP2s	Reverse mutation assay	5–10 mM	– S9 mix	Dose-dependent induction of revertants	Akinshina et al. (1992)
E. coli strain TC3960 (ΔuvrB, pKM101)	Mutations in *lacI* gene	10 µM	– S9 mix	Induced mutation frequency 30 times over the spontaneous frequency. Predominantly base pair substitutions	Bertenyi and Lambert (1996)
S. Typhimurium TA 100, TA98	Reverse mutation assay	0.01–10 µg/plate	± S9 mix	Furazolidone caused dose-dependent induction of revertants (tested only in TA100)	Vroomen et al. (1987a)
S. Typhimurium TA 98, TA 98NR, TA 98/1,8-DNP_6	Reverse mutation assay	0.1–2.5 µg/plate	± S9 mix	The highest dose was toxic in all strains. Positive in all strains with and without S9 mix	Ni et al. (1987)
Drosophila melanogaster	Sex-linked recessive lethal test	0.18, 0.44 or 0.5 mM in DMSO	± S9 mix	Positive	Kramers (1982)
CHO cells	Gene mutation assay	up to 125 µg/mL/5 hours	± S9 mix	Positive. Toxic > 100 µg/mL	Gao et al. (1989)
Isolated human lymphocytes	*In vitro* chromosomal aberration assay and UDS	0.5–100 µM	– S9 mix	Negative. No data on toxicity	Tonomura and Sasaki (1973)
Isolated human lymphocytes	*In vitro* chromosomal aberration and SCE assay	0.2–20.0 µg/mL	– S9 mix	Significant increase in SCE at 0.2 and 2 µg/mL, and chromosome breaks at 2 µg/mL. At 20 µg/mL the cells did not enter metaphase	Cohen and Sagi (1979)
Isolated human lymphocytes	SCE	2–10 µg/mL	– S9 mix	Significant increase in SCE frequency at concentrations ≥ 4 µg/mL	Madrigal-Bujaidar et al. (1997)
Bovine and porcine	*In vitro* chromosomal	0.05–500 mg/L	– S9 mix	Positive. No toxicity was observed	Queinnec et al.

Test organism/system	Method	Concentration/ Treatment	Metabolic activation	Outcome	Reference
lymphocytes	aberration assay				(1975); Babile et al. (1978)
Human lymphoblastoid cell line (TK6)	Comet assay	20–60 µg/mL	– S9 mix	Significant increase in percentage tail DNA, tail length and olive tail moment. No dose response	Borroto et al. (2005)
Human hepatoma cells HepG2	Comet assay	12.5, 25, and 50 µg/mL (3 hours)	– S9 mix	Significant increase in OTM at all concentrations. Induction of ROS formation. 8-hydroxydeoxyguanosine staining revealed oxidative damage of nuclear and mitochondrial DNA	Jin et al. (2011)
Isolated human lymphocytes	*In vitro* micronucleus assay	0.001–10 µM Synthetic free form	– S9 mix	Significant increase in micronucleus frequency at all concentrations	Barragán Hernández et al. (2011)
Swiss CD-1 mice	*In vivo* micronucleus assay	300 mg/kg b.w. in methyl cellulose		Negative. Toxicity was observed	Enninga and Weterings (1990) as cited by FAO/WHO (1993c)
Swiss Webster mice	*In vivo* micronucleus assay	100 and 500 mg/kg b.w. p.o.		Equivocal	Paik (1985)
Mice	Bone marrow SCE assay	8.6, 30.0, or 75.0 mg/kg b.w.		Significant increase in SCE frequency at concentrations ≥ 30 mg/kg b.w.	Madrigal-Bujaidar et al. (1997)

CHO: Chinese hamster ovary; DMSO: dimethyl sulphoxide; OTM: olive tail moment; SCE: sister chromatid exchange; ROS: reactive oxygen species; UDS: unscheduled DNA synthesis.

Table H.2: *In vitro* and *in vivo* genotoxicity studies of the furazolidone metabolite 3-amino-2-oxazolidinone (AOZ)

Test organism/system	Method	Concentration/ treatment	Metabolic activation	Outcome	Reference
DNA adduct formation in mice liver *in vivo*	Electrochemical detection of 7-methylguanine and O6-methylguanine	32, 125, 500 mg/kg b.w. 4 hours		An increase in the frequency of 7-methyguanine, but no O6-methylguanine	deGroot and van Zeeland (1994)
Salmonella Typhimurium TA1537, TA98, TA1535, TA100; *Escherichia coli* WP2uvrA	Reverse mutation assay	100–5 000 µg/plate	± S9 mix	In the absence of S9 mix, AOZ was positive in TA1535. In the presence of S9 mix, it was positive in TA1535, TA100 and WP2uvrA (GLP)	NOTOX (1994f)
S. Typhimurium TA98, TA100, TA1535, TA1537	Reverse mutation assay	0.1–5 mg/plate	± S9 mix	Positive in TA1535 and TA100 in the presence of metabolic activation. In the absenceof metabolic activation, weak response only in TA1535	Hoogenboom et al. (2002)
Isolated human lymphocytes	*In vitro* micronucleus assay	0.0001–0.01 µM	– S9 mix	AOZ induced significant increase in micronucleus frequency at 0.01 µM	Barragán Hernández et al. (2011)
Isolated human lymphocytes	*In vitro* chromosomal aberration assay	1 000, 3 330, 5 000 µg/mL fixed after 24 hours, 5000 µg/mL fixed after 48 hours	± S9 mix	Dose-dependent induction of chromosomal aberrations only in the absence of S9 mix (GLP)	NOTOX (1994d)
Isolated human lymphocytes	*In vitro* chromosomal aberration assay	0.33–5 mg/mL	± S9 mix	Dose-dependent induction of chromosomal aberrations only in the absence of S9 mix	Hoogenboom et al. (2002)
Swiss mice (OF-1 SPF-quality; 7 weeks old)	*In vivo* micronucleus assay	Male 500, 1 000 mg/kg b.w.; female 1 000, 2 000 mg/kg b.w.; single i.p. administration sampling at 24 and 48 hours		Positive. High mortality was observed at the higher doses. At lower doses statistically significant increase of micronucelated PCE was detected only at 24-hour sampling. The animals treated with the lower dose were lethargic (GLP)	NOTOX (1994g)
Swiss mice (OF-1, SPF-quality; 7 weeks old)	*In vivo* micronucleus assay	Male: 32–500 mg/kg b.w.; female 250–1 500 mg/kg b.w.; single i.p. administration, sampling at 24 and 48 hours		Positive: Statistically significant increase in micronucelated PCE was detected only at 48-hour sampling in the highest dose group males. Large differences in toxicity and mortality were observed indicating possible non-genotoxic mechanism (GLP)	NOTOX (1994h)

b.w.: body weight; GLP: good laboratory practice; PCE: polychromosome erythrocyte.

Table H.3: *In vitro* and *in vivo* genotoxicity studies of nitrofurantoin

Test organism/system	Method	Concentration/ Treatment	Metabolic activation	Outcome	Reference
Escherichia coli B/r and nrf 270	Single DNA strand breaks	50 µg/mL	– S9 mix	Positive in B/r strain, negative in nrf270 strain that lacks nitrofurazone reductase activity	McCalla et al. (1971)
E. coli B/r rec+, RecA and RecB; *Salmonella* Typhimurium TA1978, TA1538	DNA repair test	0.5 µM	– S9 mix	Positive in *E. coli* and in *S.* Typhiumurium	Yahagi et al. (1974)
E. coli WP2; *S.* Typhimurium TA1535, TA1536, TA1537, TA1538	Reverse mutation assay	0.003–0.3 µM	– S9 mix	Positive in *E. coli*; negative in *S.* Typhimurium	Yahagi et al.(1974)
S. Typhimurium TA100, TA100FR	Reverse mutation assay	0.3, 3 µg/plate. Urine of rats fed with feed containing 0.5 % nitrofurantoin	– S9 mix	Positive. Urine positive	Wang and Lee (1976)
E. coli WP2, WP2uvrA	Reverse mutation assay	10, 100 µg/plate	± S9 mix	Positive. The WPuvrA strain was more sensitive than the wild-type WP2	McCalla and Voutsinos (1974) ; Setnikar et al. (1976); Lu et al. (1979)
E. coli WP2, EE97 (WP2pkM101); *S.* Typhimurium TA97,	Reverse mutation assay (fluctuation assay)	Below MIC: WP2 0.2; 0.4 µg/mL; EE97 0.1, 0.2 µg/mL; TA97: 0.32; 0.64 µg/mL	– S9 mix	Positive in all strains below the MIC	Obaseiki-Ebor and Akerele (1986)
S. Typhimurium TA98, TA100, TA1535, TA1538	Reverse mutation assay	0.025–5 µg/plate	± S9 mix	Positive in TA98 and TA100. Negative in TA1535 and TA1538	Goodman et al. (1977); DeFlora, (1979)
S. Typhimurium TA100, TA100FRI	Reverse mutation assay	2 and 10 µg/plate	± S9 mix	Positive in TA100, negative in nitroreductase deficient strain TA100FRI	Rosenkranz and Speck (1976)
S. Typhimurium TA98, TA100; TA1535, TA1537, TA1538	Reverse mutation assay	No information	± S9 mix	Positive in TA100 and TA98 (potency 775 revertants/nmol); negative in TA1535, TA1537 and TA1538	DeFlora et al. (1984)

Table continued overleaf.

Table H.3: *In vitro* and *in vivo* genotoxicity studies of nitrofurantoin (continued)

Test organism/system	Method	Concentration/ Treatment	Metabolic activation	Outcome	Reference
S. Typhimurium TA98, TA98NR, TA98/1,8-DNP_6	Reverse mutation assay	1–100 µg/plate	± S9 mix	The highest dose was toxic in all strains. Positive in all strains with and without S9 mix	Ni et al. (1987)
Human lymphoblastoid cell line (TK6)	Comet assay	2–15 µg/mL	– S9 mix	Significant increase in percentage tail DNA, Tail length and olive tail moment. No dose response	Borroto et al. (2005)
Saccharomyces cerevisiae D4, D4-RDII and D7	Mitotic gene conversion	2–42 mM (estimated from graph)	– S9 mix	Nitrofurantoin induced mitotic gene conversion in strains D4-RDII and D7, but not in strain D4	Setnikar et al. (1976); Siebert et al. (1979); Callen (1981)
Aspergillus nidulans	Non-disjunction and mitotic crossing-over (spot test)	No information	– S9 mix	Positive for both endpoints	Bignami et al. (1974)
S. cerevisiae a host mediated assay with mice	Mitotic gene conversion	72 mg/kg b.w. p.o.		Negative	Siebert et al. (1979)
S. cerevisiae D4-RDII in a host-mediated assay with rats	Mitotic gene conversion	500 mg/kg b.w. p.o.	– S9 mix	Positive	Setnikar et al. (1976)
Drosophila melanogaster	Sex-linked recessive lethal test	0.9 mM in DMSO		Negative	Kramers (1982)
CHO cells	Gene mutation assay	Up to 200 µg/mL/5 hours	± S9 mix	Nitrofurantoin induced mutations to 6-thioguanine resistance in the presence, but not in the absence, of S9 mix	Gao et al. (1989)
Rat liver nuclei and human foreskin fibroblasts (HuF22)	DNA fragmentation *in vitro*	112 mg/mL 30 minutes	– S9 mix	Positive in rat liver nuclei and in HuF22 cells	Parodi et al. (1983)
Human lymphoblastoid cell line (TK6)	Comet assay	2–15 µg/mL	– S9 mix	Significant increase in percentage tail DNA, Tail length and olive tail moment. No dose response	Borroto et al. (2005)
Isolated human lymphocytes	*In vitro* chromosomal aberrations and UDS	1–100 µM	– S9 mix	Negative for both endpoints	Tonomura and Sasaki (1973)

Table continued overleaf.

Table H.3: *In vitro* and *in vivo* genotoxicity studies of nitrofurantoin (continued)

Test organism/system	Method	Concentration/ Treatment	Metabolic activation	Outcome	Reference
Isolated human lymphocytes	*In vitro* chromosomal aberrations and SCE	5, 20, 40 µM 24 hours	– S9 mix	A significant increase of chromatide-type chromosomal aberrations and SCE at 20 and 40 µM	Slapsyte et al. (2002)
NMRI mice	*In vivo* chromosomal aberration in spermatocytes	8 or 40 mg/kg b.w. i.p. for 5 days		Negative	Fonatsch (1977)
Mice	Dominant lethal test	16 and 80 mg/kg b.w. i.p.		Negative	Epstein et al. (1972)
Sprague–Dawley rats	*In vivo* micronucleus assay	50, 100, 200 mg/kg b.w. i.p.—half dosed 30 hours and half 6 hours before sacrifice		Negative. No evidence that the compound reached target tissue is given. Positive control thiethylenemelamine gave positive response	Goodman et al. (1977)
Sprague–Dawley rats, Swiss mice	*In vivo* DNA fragmentation	14–112 mg/kg b.w. i.p. Sacrificed between 6 and 168 hours after administration		DNA fragmentation in liver, spleen, kidney, lung, bone marrow. In kidney also at 14 mg/kg b.w.	Parodi et al. (1983)
Swiss mice	*In vivo* bone marrow SCE	32, 64 mg/kg b.w. i.p. Sampling 24 and 72 hours after administration		Dose-dependent increase in SCE frequency	Parodi et al. (1983)
BALB/C mice (3 and 8 weeks old)	*In vivo* micronucleus assay	5, 10, 50 mg/kg b.w.; single i.p. injection before and 48, 96, 168 and 336 hours after administration		Significant increase in the frequency of micronucleus at all doses. In old but not young animals the micronucleus frequency dropped to the frequency before administration	Fucić et al. (2005)
Male Big BlueTM C57BL/6[LIZ] mice	Transgenic mouse mutation assay	167 mg/kg b.w.; p.o five consecutive days/20 days after last administration		Frequency of *cII* gene mutations from the shuttle vector was determined in different organs. More than 1.5-fold mutant frequency increase only in kidney	Quillardet et al. (2006)

Table continued overleaf.

Table H.3: *In vitro* and *in vivo* genotoxicity studies of nitrofurantoin (continued)

Test organism/system	Method	Concentration/ Treatment	Metabolic activation	Outcome	Reference
Blood lymphocytes of treated children	*In vivo* chromosomal aberrations and SCE	Prophylactic treatment 5–8 mg per day for 7 days and 1–2 mg per day for rest of the treatment period. Sampling: before the start and at 1, 3, 6 and 12 months of therapy		A significant correlation between cumulative dose and SCE frequency was observed after 1 month of therapy but not after 12 months. The increase in the rate of chromosomal aberrations was ascribed to the X-ray examination prior to the treatment	Slapsyte et al. (2002)
Blood lymphocytes of treated women	*In vivo* SCE	Urinary tract infection treatment 10 or 400 mg per day for 10 days. Sampling before and after treatment		Negative	Sardas et al. (1990)

b.w.: body weight; CHO: Chinese hamster ovary; DMSO: dimethyl sulphoxide; i.p.: intraperitoneal; MIC: minimum inhibitory concentration; p.o.: *per os*; SCE: sister chromatid exchange.

Table H.4: *In vitro* and *in vivo* genotoxicity studies of nitrofurazone

Test organism/system	Method	Concentration/ treatment	Metabolic activation	Outcome	Reference
Eschericihia coli B/r and nrf 207	Single DNA strand breaks	25, 50 µg/mL	– S9 mix	Positive in B/r strain negative in nrf207 strain that lacks nitrofurazone reductase activity	McCalla et al. (1971)
E. coli WP2, WP2uvrA, nrf343 and nrf345	Reverse mutation assay	10–100 µg/plate	± S9 mix	Positive. Negative in nitrofurazone reductase activity deficient nrf343 and nrf345 strains	McCalla and Voutsinos (1974)
E. coli WP2, WP2uvrA	Reverse mutation assay	50–250 µM	– S9 mix	Positive. The WPuvrA strain was more sensitive than the wild-type WP2	Lu et al. (1979)
Salmonella Typhimurium TA1535, TA100; *E. coli* WP2uvrA	Reverse mutation assay (fluctuation assay)	100 ng/mL (TA1535; WP2uvrA) 0.01–1 ng/mL (TA100)	– S9 mix	Positive. In TA100 significant induction at concentration ≥ 0.1 ng/mL	Green et al. (1977)
S. Typhimurium TA1535, TA1975; TA1536, TA1537, TA1538, TA1976, TA1977, TA1978; *E. coli* WP2uvrA	Reverse mutation assay	0.5–5 µg/plate	– S9 mix	Negative in *S.* Typhimurium strains, positive in *E. coli* strain	Mc Calla et al. (1975)
E. coli WP2, EE97 (WP2 pkM101); *S.* Typhimurium TA 97	Reverse mutation assay (fluctuation assay)	Below MIC: WP2 0.04; 0.08 µg/mL; EE97: 0.02, 0.04 µg/mL; TA97: 0.32; 0.64 µg/mL	– S9 mix	Positive in all strains below MIC	Obaseiki-Ebor and Akerele (1986)
E. coli B/r rec+, RecA and RecB; *S.*Typhimurium TA1978, TA1538	DNA repair test	0.5 µM	– S9 mix	Positive in *E. coli*; negative in *S.* Typhiumurium	Yahagi et al. (1976)
S. Typhimurium TA1535, TA100	Reverse mutation assay	0.1–1 µg/plate	– S9 mix	Negative in *S.* Typhiumurium TA1535; positive in TA100	Yahagi et al. (1974)
S. Typhimurium TA98, TA100, TA1535, TA1538	Reverse mutation assay	0.025–5 µg/plate	± S9 mix	Positive in TA98 and TA 100, negative in TA1535 and TA1538	Goodman et al. (1977)
Aspergillus nidulans	Forward mutation to 8-azaguanine resistance and induction of mthA1 repressors	100–1000 µg/mL	– S9 mix	Negative	Bignami et al. (1974)
Neurospora crassa	Ad-3 mutation induction	1 mM	– S9 mix	Positive	Ong (1977)
Drosophila melanogaster	Sex-linked recessive lethal test	5 mM in DMSO		Negative	Kramers (1982)

Test organism/system	Method	Concentration/ treatment	Metabolic activation	Outcome	Reference
Mouse L929, Hamster BHK-21, human KB cells	DNA strand breaks	125–500 µM 90 minutes	– S9 mix	Positive	Olive and McCalla (1975)
Human lymphoblastoid cell line (TK6)	Comet assay	5–30 µg/mL	– S9 mix	Significant increase in percentage tail DNA, tail length and olive tail moment. No dose response	Borroto et al. (2005)
Chinese hamster lung cells (CHL)	Chromosomal aberrations	0.1 mg/mL	± S9 mix	Negative without metabolic activation, positive with metabolic activation	Matsuoka et al. (1979)
Isolated human lymphocytes	*In vitro* chromosomal aberrations and UDS	1–100 µM	– S9 mix	Negative for both endpoints	Tonomura and Sasaki (1973)
CHO cells	Chromosomal aberrations, *HGPRT* mutations	25–200 µg/mL for 2 hours	± S9 mix	Positive for chromosomal aberrations. Weak mutagenic response without dose response	Anderson and Philips (1985)
Male Wistar rats	Chromosomal aberrations in bone marrow	Single dosage 40, 120, 400 mg/kg b.w. sampling after 6, 24, 48 hours: five daily doses of 15, 45 or 150 mg/kg b.w. sampling 6 hours after the last dosage		Negative. Mitotic index was decreased at 400 mg/kg b.w. The positive control EMS gave positive response in both treatment schedules	Anderson and Philips (1985)
Sprague–Dawley rats	*In vivo* micronucleus assay; chromosomal aberrations	15, 30, 60 mg/kg b.w. i.p.—half dosed 30 hours and half 6 hours before sacrifice		Negative. No evidence that the compound reached target tissue is given. Positive control thiethylenemelamine gave positive response	Goodman et al. (1977)

b.w.: body weight; CHL: Chinese hamster lung; CHO: Chinese hamster ovary; DMSO: dimethyl sulphoxide; EMS: ethyl methanesulphonate; i.p.: intraperintoneal; MIC: minimum inhibitory concentration; UDS: unscheduled DNA synthesis.

Table H.5: *In vitro* and *in vivo* genotoxicity studies of semicarbazide

Test organism/system	Method	Concentration/ treatment	Metabolic activation	Outcome	Reference
Salmonella Typhimurium G46, C3076, D3052, TA1535, TA1537, TA1538, TA98, TA100; *Eschericihia coli* WP2, WP2uvrA	Reverse mutation assay—modified protocol	No data	± S9 mix	Negative	McMahon et al. (1979)
S. Typhimurium TA1535, TA1537, TA1538, TA98, TA100	Reverse mutation assay	No data	± S9 mix	Positive only in TA1535; the activity decreased in the presence of metabolic activation	DeFlora et al. (1981); DeFlora et al. (1984)
S. Typhimurium TA1535, TA1537, TA98, TA100 and TA102	Reverse mutation assay	50–7 000 µg/plate	± S9 mix	Positive only in TA1535 at doses ≥ 5000 µg/plate only in the absence of metabolic activation (GLP)	Herbold (2003)
S. Typhimurium TA1535, TA1537, TA98 and TA100, and *E. coli* WP2uvrA	Reverse mutation assay	62–5 000 µg/plate	± S9 mix	Dose-dependent increase of revenants in TA1535. Higher activity without metabolic activation. Borderline activity in TA100 without metabolic activation	TNO (2004a)
L5178Y cells	*In vitro* forward mutation assay at *tk* locus	0.21–10.0 mM	± S9 mix	Positive without metabolic activation. With metabolic activation slight increase of mutant colonies at the highest concentration	TNO (2004b)
CHO cells	*In vitro* chromosomal aberration	75–1 115 µg/mL, 4, 18, 32 hours exposure; sampling after 18 and 32 hours	± S9 mix	Negative for chromosomal aberration. With metabolic activation after 18 hours increase in endoreduplicated cells	TNO (2004c)
Chinese hamster V79 cells	*In vitro* chromosomal aberration	125–1 120 µg/mL sampling after 4 and 18 hours	± S9 mix	Negative (GLP)	Herbold (2004)
Isolated human lymphocytes	*In vitro* micronucleus and SCE assays	0.5–20 µg/mL	– S9 mix	No significant increase in micronucleus and SCE frequency over the control	Vlastos et al. (2010)
Male mice Balb/C and CBA	*In vivo* micronucleus (flow-cytometry determination)	Single i.p. dose 40, 80 or 120 mg/kg b.w.; blood sampling after 42 hours		Negative: no increase in micronucleus frequency, no suppression of the percentage PCE. The positive control, colchicine, gave a positive response	Abramsson-Zetterberg and Svensson (2005)
Female CD-1 mice	*In vivo* UDS in liver	Single p.o. dose 100 or 200 mg/kg b.w.; sampling		Negative. Positive controls induced marked increase in UDS compared with vehicle	CTL (2004)

Test organism/system	Method	Concentration/ treatment	Metabolic activation	Outcome	Reference
		after 4 and 16 hours		control (GLP)	
Male Wistar rats (5–6 weeks of age)	*In vivo* micronucleus assays	Single p.o. dose 50, 100, 150 mg/kg b.w./sampling after 24 hours		Significant, > twofold increase in micronucleus frequency over the control at all doses without dose–response pattern	Vlastos et al. (2010)

b.w.: body weight; CHO: Chinese hamster ovary; GLP: good laboratory practice; p.o.: *per os*; PCE: polychromosome erythrocyte; SCE: sister chromatid exchange; UDS: unscheduled DNA synthesis.

Table H.6: *In vitro* and *in vivo* genotoxicity studies of nifursol

Test organism/system	Method	Concentration/ treatment	Metabolic activation	Outcome	Reference
DNA binding in rat tissue *in vivo*		200 mg/kg b.w, radiolabelled nifursol. Binding was measured in liver, kidney and intestine after 6 and 24 hours		Weak radioactivity associated with DNA was detected in liver, kidney and intestines. It cannot be excluded that DNA-associated radioactivity represents a nifursol impurity or a minor nifursol metabolic product incorporated into DNA (GLP)	Connelly (1988)
Salmonella Typhimurium TA1535, TA1537, TA1538, TA98, TA100	Reverse mutation assay	0.2–20 µg/plate	± S9 mix	Negative. However, the rates of spontaneous reverse mutations are not typical of the test strains used	Green (1980)
S. Typhimurium TA1535, TA1537, TA1538, TA98, TA100	Reverse mutation assay	6.7–1 500 µg/plate	± S9 mix	Positive in TA100 with and without metabolic acitivation. Positive in TA98 only with metabolic activation (GLP)	Cavagnaro and McCarrol (1985)
Chinese hamster ovary cells (CHO K-1)	*In vitro* chromosomal aberration	2.5, 8.8, 25, 85 and 250 µg/mL, 2 hours exposure, sampling after 10 hours	± S9 mix	Significantly elevated number of chromosomal aberrations at 85 µg/mL without metabolic activation. At higher concentrations precipitate was formed and was not analysed	Cavagnaro and Cortina (1985a)
CHO cells	*In vitro* chromosomal aberration	50, 75, 100, 150 and 200 µg/mL, 2 hours exposure, sampling after 10 hours	± S9 mix	Consistent but insignificant increase in chromosomal aberration was observed at the maximal soluble concentration without metabolic activation. (GLP)	Cavagnaro and Cortina (1985b)
Isolated rat hepatocytes	*In vitro* UDS	1, 5, 10, 50, 100, 500 µg/mL		Negative. Small increases were observed but none was significant and without dose response. (GLP)	Cavagnaro and Sernau (1985)
Male and female mice	*In vivo* bone marrow micronucleus assay	Single dose 10 000 mg/kg b.w. by gavage; sampling after 24, 42 and 72 hours		Negative. No significant increase in the frequency of MN PMC; no decrease in the ratio of PMC to NMC. Positive control (mitomicyn C) gave clear positive response	Allen and Proudlock (1987)
Male and female rat	*In vivo* bone marrow chromosomal aberration assay	Single dose 10 000 mg/kg b.w. by gavage; sampling after 6, 24 and 48 hours		Negative. No significant increase in the frequency of cells with chromosomal aberrations. The positive control, cyclophosphamide caused significant increase in the frequency of cells with chromosomal aberrations. (GLP)	Allen et al. (1987)

Test organism/system	Method	Concentration/ treatment	Metabolic activation	Outcome	Reference
Male Wistar rats	*In vivo* UDS in liver and intestinal cells	Single dose 100, 300 or 1 000 mg/kg b.w. by gavage; sampling after 2 and 12 hours		Negative in liver cells. In intestinal cells UDS was induced at 300 or 1 000 mg/kg b.w. after 12 hours. Statistically significant only in the 300 mg/kg b.w. group. Irritation of intestinal tissue was observed. (GLP)	Benford (1987a)
Male Wistar rats	*In vivo* UDS in intestinal and gut cells	Single dose 100, 300 or 1 000 mg/kg b.w. by gavage; sampling after 2 and 12 hours		Increased incorporation of tritiated thymidine at 300 or 1 000 mg/kg b.w. after 12 hours. Statistically significant only in the 300 mg/kg b.w. group. Irritation of intestinal tissue was observed (GLP)	Benford (1987b)
Muta-Mice	Transgenic mouse mutation assay	550 or 850 mg kg b.w. for 28 days p.o., necropsy at day 31		Frequency of *lacZ*gene mutations from the shuttle vector was determined in the ileum/jejunum. No increase was observed (GLP)	Ballantyne (2003)

b.w.: body weight; CHO: Chinese hamster ovary; GLP: good laboratory practices; NMC : normochromatic micronucleated erythrocytes; PMC : polychromatic micronucleated erythrocytes; p.o.: *per os*; UDS: unscheduled DNA synthesis.

Appendix I. Benchmark dose analyses

The results of the BMD analyses are reported in general as follows:

Quantal endpoints. The dataset analysed and the results of the analyses using five quantal dose–response models (log-logistic, log-probit, Weibull, gamma, logistic) are tabulated. For each model, the log-likelihood values, whether the model was accepted or not (according to goodness-of-fit test, $p \geq 0.05$), and the BMD confidence interval (BMDL, BMDU) for the accepted models are reported. The (maximum likelihood estimates of the) BMD values are reported for the endpoints that were used in the risk characterisation (i.e. Tables I.2 (for furazolidone) and I.20 (for SEM)), except for nitrofurantoin (see Section I.5). An overall BMD confidence interval is reported based on the lowest BMDL and highest BMDU from the five models. Finally, a figure shows the dose–response data, together with one of the fitted models (the log-logistic in each case). The BMD analysis for the effect of furaltadone on mammary tumours is reported in a concise way because the analysis was not used in the risk assessment due to the large difference between the BMDL and BMDU.

Continuous endpoints. The dataset analysed is tabulated. The results of the fitted (four-parameter) exponential and Hill model are shown in figures for all endpoints and in tables for the endpoints used for the risk characterisation. The legends of the figures provide details, including parameter estimates, and the established BMDL, BMD and BMDU. An overall BMD confidence interval is reported based on the lowest BMDL and highest BMDU from the two models.

Note that these overall BMD confidence intervals reflect intervals with a greater confidence than the 90 % level related to the confidence intervals for the individual models (due to the extreme values calculated for the different models used), in particular in the case of quantal data where five models were used.

A benchmark response (BMR) of 10 % was used for quantal data and 5 % for continuous data as recommended by the EFSA Scientific Committee (EFSA, 2009).

Uncertainty in BMD calculations may be reflected by the width of the confidence interval (BMDL to BMDU), or by the BMDU/BMDL ratio. Therefore, BMDL and BMDU values are reported for all the BMD analyses shown and BMD values are only reported for those endpoints that were used in the risk characterisation.

Accounting for the uncertainty in the BMD calculation in each of these analyses, the CONTAM Panel noted that for the endpoints used to characterise the risk, the BMDU/BMDL ratio (from the overall cofindence interval (CI)) did not exceed one order of magnitude. The BMD analyses for osteosarcomas caused by nitrofurantoin, resulted in a BMDU of infinity (see Section I.5) and, therefore, no BMDU/BMDL ratio was calculated.

I.1. Furazolidone: carcinogenicity endpoints

The CONTAM Panel considered the tumour data from four carcinogenicity studies suitable for dose–response modelling:

1. Halliday et al. (1974): bronchial adenocarcinomas observed in male and female Swiss MBR/ICR mice;
2. King et al. (1972a); Halliday et al. (1973a): malignant mammary tumours (adenocarcinomas and carcinosarcomas) observed in female Sprague–Dawley rats;
3. King et al. (1972b); Halliday et al. (1973b): mammary adenocarcinomas observed in Fischer 344 rats;

4. King et al. (1972b); Halliday et al. (1973b): malignant mammary tumours (adenocarcinomas and carcinosarcomas) in female and neural astrocytomas observed in male Sprague–Dawley rats.

The BMDL and BMDU that are derived from these four studies should be considered to be indicative, because all four studies had mortality before the end of the study. The animals that died prematurely without tumours might have developed tumours (in response to the dose) had they not died. This results in additional uncertainty in the estimated dose–response relationship, which is not covered by the reported BMD confidence intervals.

I.1.1. Bronchial adenocarcinomas in mice

The data for bronchial adenocarcinomas in mice from Halliday et al. (1974) are given in Table I.1. The data from both male and female animals were combined. The dose–response analysis revealed no significant differences between both genders, thus the results hold equally for both males and females.

Table I.1: Dose–response data for bronchial adenocarcinomas from Halliday et al. (1974)

Dose (mg/kg b.w. per day)	Number of animals	Number of animals with bronchial adenocarcinomas	Sex
0	49	13	M
12	48	19	M
24	50	26	M
47	50	37	M
0	50	15	F
12	50	18	F
24	47	20	F
47	48	30	F

b.w.: body weight; F: female; M, male.

As Table I.2 shows, the lowest $BMDL_{10}$ was 3.5 mg/kg b.w. per day and the highest $BMDU_{10}$ was 22 mg/kg b.w. per day, resulting in an overall BMD confidence interval of 3.5–22 mg/kg b.w.

The results in Table I.2 were re-calculated by the BMDS software, resulting in the same values for the $BMDL_{10}$. (Note: the BMDS software does not provide for combining dose–response data from different subgroups differing in one or more model parameters, but in this case the two subgroups were assumed to have identical dose–response curves.)

The data for both genders were also analysed separately. The overall BMD_{10} confidence interval for males was 1.2–21 mg/kg b.w. per day and for females 2.0–48 mg/kg b.w. per day. These values are in line with the results reported by Carlsson Forslund (2014).

Table I.2: Benchmark dose analysis[(a)] for bronchial adenocarcinoma

Model	Number of parameters	Log-likelihood	Accepted	$BMDL_{10}$ (mg/kg b.w. per day)	BMD_{10}[(b)] (mg/kg b.w. per day)	$BMDU_{10}$[(b)] (mg/kg b.w. per day)
Null	1	–270.06	-			
Full	8	–250.86	-			
Two.stage	3	–252.2	Yes	5.23	9.86	19.4
Log.logist	3	–252.27	Yes	4.49	11.4	22.2
Weibull	3	–252.22	Yes	3.75	10.5	21.9
Log.prob	3	–252.32	Yes	4.96	11.7	22.4
Gamma	3	–252.23	Yes	3.47	10.7	22
Logistic	2	–252.2	Yes	7.8	9.24	11.5

b.w.: body weight.
(a): Covariate: sex; BMR: 0.1 extra risk; constraint: no; p-value goodness of fit: 0.05.

(b): The BMDL and BMDU values should be considered to be indicative.

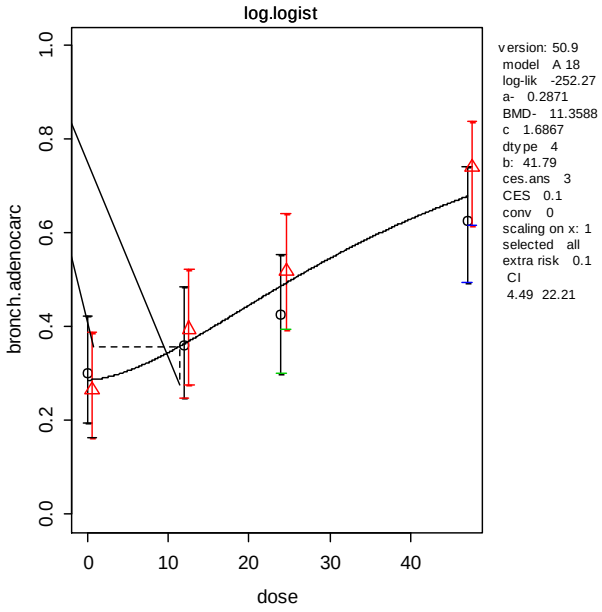


Figure I.1: The dose–response data for the bronchial adenocarcinomas (triangles: males; circles: females) with the fitted log-logistic model, assumed to be identical for both sexes based on the statistical analysis of the two dose responses. The two dashed lines indicate the benchmark response of 10 % and the associated benchmark dose for this curve. Note that the data for males were slightly shifted to the right, to visually distinguish the confidence intervals for the responses.

I.1.2. Malignant mammary tumours

The mammary tumours as reported in two studies in Sprague–Dawley rats (Table I.3; Halliday et al., 1973a and Halliday et al., 1973b) were combined in the BMD analysis. Only the background response was found to differ significantly between both studies. Therefore, both studies estimate the same value for the BMD_{10}. The overall BMD_{10} confidence interval was 25–86 mg/kg b.w. per day (Table I.4).

Table I.3: Data on malignant mammary tumours (adenocarcinomas and carcinosarcomas) from two carcinogenicity studies as used for the benchmark dose analysis

Dose (mg/kg b.w. per day)	Number of animals	Number of animals with mammary tumours	Reference
0	35	1	Halliday et al. (1973a)
0.8	35	3	Halliday et al. (1973a)
4.3	35	4	Halliday et al. (1973a)
14	35	4	Halliday et al. (1973a)
0	50	1	Halliday et al. (1973b)
12.5	50	1	Halliday et al. (1973b)
25	50	3	Halliday et al. (1973b)
50	50	8	Halliday et al. (1973b)

b.w.: body weight.

Table I.4: Benchmark dose (BMD) analysis[(a)] for mammary tumours, in which study was included as a covariate. The $BMDL_{10}$s and $BMDU_{10}$s hold similarly for both studies, as only parameter a (background response) was found to differ between the studies.

Model	Par.covar[(c)]	Number of parameters	Log-likelihood	Accepted	$BMDL_{10}$[(d)] (mg/kg b.w. per day)	$BMDU_{10}$[(d)] (mg/kg b.w. per day)
Null		1	–89.31	–		
Full		8	–82.51	–		
Two.stage	a[(b)]	4	–83.84	Yes	25.4	72.8
Log.logist	a[(b)]	4	–83.85	Yes	25.1	82.9
Weibull	a[(b)]	4	–83.85	Yes	25.3	82.2
Log.prob	a[(b)]	4	–83.85	Yes	24.8	86
Gamma	a[(b)]	4	–83.85	Yes	25.3	81.8
Logistic	a[(b)]	3	–83.7	Yes	33.6	60.9

b.w.: body weight; $BMDL_{10}$: lower 95 % confidence limit for a benchmark response of 10 % extra risk; BMDU:benchmark dose upper confidence limit.
(a): BMR: 0.1 extra risk; constraint: no; p-value goodness of fit: 0.05.
(b): a = background response parameter in the dose–response model.
(c): Par.covar = model parameter(s) that were found to differ significantly between the subgroups (studies).
(d): The BMDL and BMDU values should be considered to be indicative.

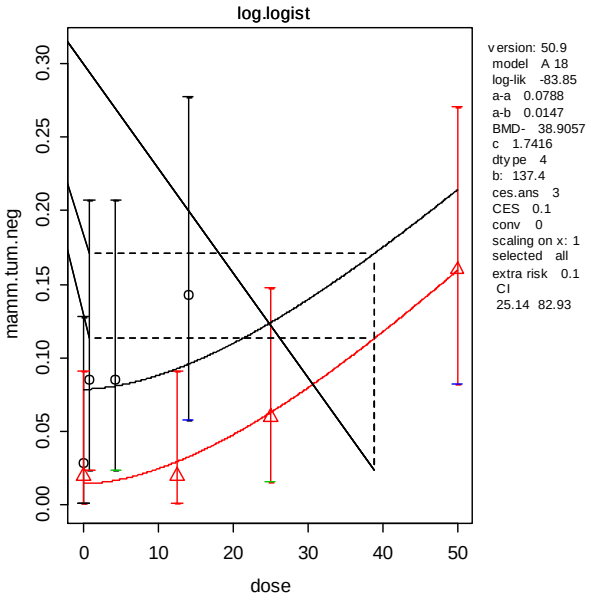


Figure I.2: The dose–response data for the malignant mammary tumours (triangles: Halliday et al., 1973b; circles: Halliday et al., 1973a) with the fitted log-logistic model, assumed to differ in the background response but not in sensitivity to the compound, nor in shape, based on the statistical analysis of the two dose responses. The two horizontal dashed lines indicate the benchmark response of 10 % for each study, the vertical dashed line the associated benchmark dose for these curves (which holds for both datasets in this case).

I.1.3. Mammary adenocarcinomas

Mammary adenocarcinomas were reported in both studies with Sprague–Dawley rats, as well as in the study with Fisher 344 rats (Table I.5; Halliday et al., 1973a; Halliday et al., 1973b). The data from these three studies were combined (for females only). No significant differences in dose–response among studies were found other than in the background response. The overall BMD_{10} confidence interval was 37–60 mg/kg b.w. per day (Table I.6).

Table I.5: Data on mammary adenocarcinomas from three carcinogenicity studies as used for the benchmark dose analysis

Dose (mg/kg b.w. per day)	Number of female animals	Number of female animals with mammary adenocarcinoma	Rat strain	Reference
0	34	1	Sprague–Dawley	Halliday et al. (1973a)
0.8	35	2	Sprague–Dawley	Halliday et al. (1973a)
4.3	33	2	Sprague–Dawley	Halliday et al. (1973a)
14	35	3	Sprague–Dawley	Halliday et al. (1973a)
0	49	1	Sprague–Dawley	Halliday et al. (1973b)
12.5	50	0	Sprague–Dawley	Halliday et al. (1973b)
25	50	3	Sprague–Dawley	Halliday et al. (1973b)
50	50	8	Sprague–Dawley	Halliday et al. (1973b)
0	49	0	Fischer 344	Halliday et al. (1973b)
12.5	50	0	Fischer 344	Halliday et al. (1973b)
25	50	0	Fischer 344	Halliday et al. (1973b)
50	50	6	Fischer 344	Halliday et al. (1973b)

b.w.: body weight.

Table I.6: Benchmark dose (BMD)[(a)] results for mammary adenocarcinomas, in which study was included as a covariate. The BMDLs and BMDUs hold similarly for both studies, as only parameter a (background response) was found to differ between the studies.

Model	Par.covar[(b)]	No.par	Log-likelihood	Accepted	$BMDL_{10}$[(d)] (mg/kg b.w. per day)	$BMDU_{10}$[(d)] (mg/kg b.w. per day)	Sens.subgr
Null		1	–103.99	–			
Full		12	–86.52	–			
Two.stage	a[(c)]	5	–90.34	Yes	37.4	60.3	–
Log.logist	a[(c)]	5	–89.24	Yes	39.4	54.3	–
Weibull	a[(c)]	5	–89.26	Yes	40	54.4	–
Log.prob	a[(c)]	5	–89.23	Yes	38.1	55.3	–
Gamma	a[(c)]	5	–89.23	Yes	39	54.6	–
Logistic	a[(c)]	4	–88.69	Yes	43.4	53	–

b.w.: body weight; $BMDL_{10}$: lower 95 % confidence limit for a benchmark response of 10 % extra risk; BMDU:benchmark dose upper confidence limit.
(a): BMR: 0.1 extra risk; covariate: study; constraint: no; p-value goodness of fit: 0.05.
(b): Par.covar = model parameter(s) that were found to differ significantly between the subgroups (studies).
(c): a = background response parameter in the dose–response model.
(d): The BMDL and BMDU values should be considered to be indicative.

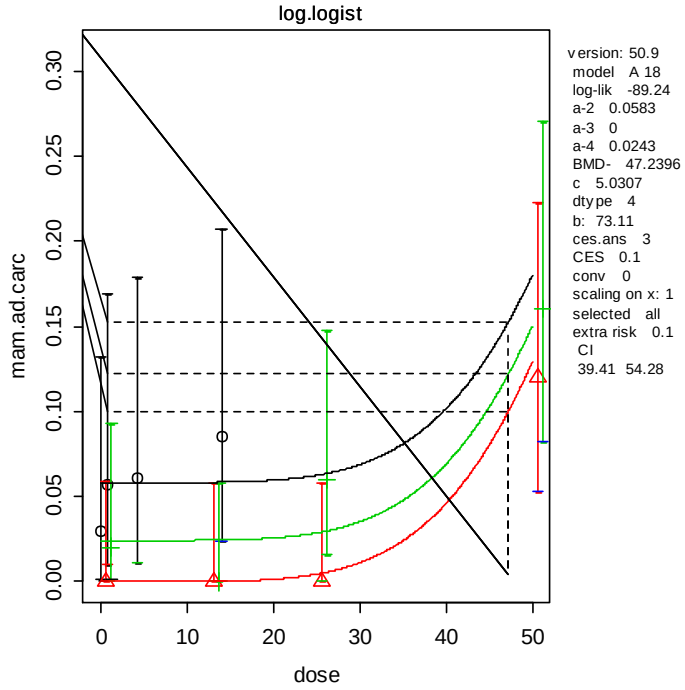


Figure I.3: The dose–response data for the mammary adenocarcinomas (triangles: Fisher rat study reported by Halliday et al., 1973b; pluses: Sprague–Dawley rat study reported by Halliday et al., 1973b; circles: Sprague–Dawley rat study reported by Halliday et al., 1973a) with the fitted log-logistic model, assumed to differ in the background response but not in sensitivity to the compound, nor in shape, based on the statistical analysis of the two dose responses. The three horizontal dashed lines indicate the benchmark response of 1 0 % for each study, the vertical dashed line the associated benchmark dose for these three curves (which holds for all three datasets in this case).

I.1.4. Astrocytomas

The astrocytomas reported in Sprague–Dawley rats (Table I.7; Halliday et al., 1973b) were analysed for the males. The overall BMD_{10} confidence interval was 35–120 mg/kg b.w. per day (see Table I.8).

Table I.7: Data on astrocytomas from one carcinogenicity study as used for the benchmark dose analysis

Dose (mg/kg b.w. per day)	Number of males	Number of males with astrocytomas	Reference
0	50	0	Halliday et al., 1973b
12.5	50	0	Halliday et al., 1973b
25	50	2	Halliday et al., 1973b
50	50	5	Halliday et al., 1973b

b.w: body weight.

Table I.8: BMD[(a)] results for of astrocytomas in male Sprague–Dawley rats

Model	No.par	Log-likelihood	Accepted	$BMDL_{10}$[(b)] (mg/kg b.w. per day)	$BMDU_{10}$[(b)] (mg/kg b.w. per day)
Null	1	–30.34	–		
Full	4	–24.65	–		
Two.stage	3	–25.14	Yes	36.7	102
Log.logist	3	–25.13	Yes	36.1	117
Weibull	3	–25.14	Yes	36.4	116
Log.prob	3	–25.03	Yes	35.2	119
Gamma	3	–25.11	Yes	36.1	112
Logistic	2	–25.65	Yes	41.2	72.3

b.w.: body weight; $BMDL_{10}$: lower 95 % confidence limit for a benchmark response of 10 % extra risk; BMDU:benchmark dose upper confidence limit.
(a): BMR: 0.1 extra risk; no covariate; constraint: no; P-value goodness of fit: 0.05.
(b): The BMDL and BMDU values should be considered to be indicative.

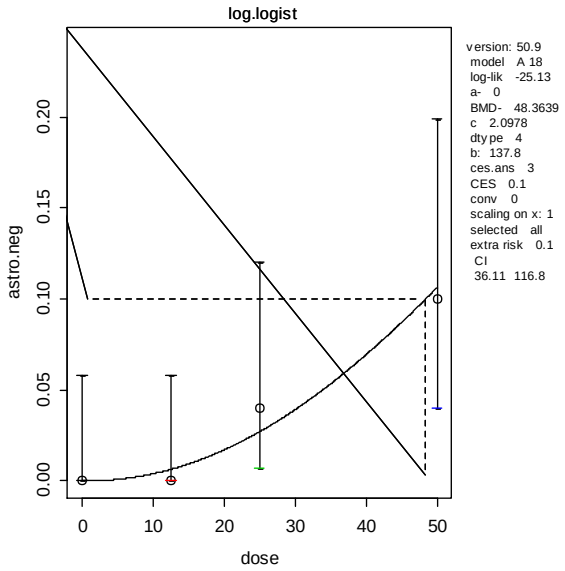


Figure I.4: The dose–response data for astrocytomas in male Sprague–Dawley rats, with the fitted log-logistic model. The dashed lines indicate benchmark response of 10 % and associated benchmark dose.

I.1.5. Summary

Table I.9 shows the BMD results for the four tumour types considered. From these results, the CONTAM Panel selected the lowest $BMDL_{10}$ value of 3.5 mg/kg b.w. per day as a reference point for the carcinogenic effects of furazolidone.

Table I.9: Summary of benchmark dose results for furazolidone

Tumour type	Study[(a)]	Single or combined dataset analysed	Species	Sex	$BMDL_{10}$[(d)] (mg/kg b.w. per day)	$BMDU_{10}$[(d)] (mg/kg b.w. per day)
Bronchial adenocarcinomas	1	Gender combined	Mice	M and F	3.5 [(b)]	22
Malignant mammary tumours	2 and 3	Studies combined	SD rats	F	25 [(c)]	86
Mammary adenocarcinomas	2 and 3	Studies combined	SD and Fisher 344 rats	F	37 [(c)]	60
Neural astrocytomas	3	Single	SD rats	M	35	120

b.w.: body weight; $BMDL_{10}$: lower 95 % confidence limit for a benchmark response of 10 % extra risk; BMDU:benchmark dose upper confidence limit; F: female; M: male;SD: Sprague–Dawley.

(a): Number refers to the following reference: (1) Halliday et al. (1974); (2) Halliday et al. (1973a); (3) Halliday et al. (1973b).

(b): Holds equally for each gender.

(c): Holds equally for each study in the dataset.

(d): The BMDL and BMDU values should be considered to be indicative.

I.2. Furazolidone: red blood cells

In both chronic studies reported by Halliday et al. (1973a;b) the number of red blood cells were measured at various points during the studies (days 360, 527 and 726 in the study reported by Halliday et al. (1973a) and days 544 and 656 in the study reported by Halliday et al. (1973b); Table I.10). The data from all five observation days were combined for BMD analysis, which resulted in significant differences in the background red blood cell values among the observation days, with a decreasing trend with age. Also the within-group variances tended to increase with age. No significant difference was found, however, for sensitivity to furazolidone among the observation days. Therefore, the calculated BMD_{05} confidence interval holds for all observation days.

Table I.10: Data on number of red blood cells in female Sprague-Dawley rats as used for the benchmark dose analysis

Day	Dose (mg/kg b.w. per day)	Number of animals	RBC ± SD	Reference
360	0	15	6.88 ± 0.43	Halliday et al. (1973a)
	0.8	15	6.59 ± 0.36	Halliday et al. (1973a)
	4.3	14	6.24 ± 0.59	Halliday et al. (1973a)
	14	15	6.17 ± 0.50	Halliday et al. (1973a)
527	0	15	6.96 ± 0.58	Halliday et al. (1973a)
	0.8	13	6.62 ± 0.53	Halliday et al. (1973a)
	4.3	10	6.55 ± 0.69	Halliday et al. (1973a)
	14	14	6.38 ± 0.67	Halliday et al. (1973a)
726	0	9	6.3 ± 1.5	Halliday et al. (1973a)
	0.8	7	6.0 ±0.5	Halliday et al. (1973a)
	4.3	6	4.9 ± 1.6	Halliday et al. (1973a)
	14	7	4.8 ±1.1	Halliday et al. (1973a)
544	0	17	6.27 ± 0.61	Halliday et al. (1973b)
	12.5	17	5.87 ± 0.88	Halliday et al. (1973b)
	25	18	5.70 ± 1.33	Halliday et al. (1973b)
	50	15	5.28 ± 0.66	Halliday et al. (1973b)
656	0	15	6.12 ± 0.72	Halliday et al. (1973b)
	12.5	15	5.83 ± 1.21	Halliday et al. (1973b)
	25	15	4.86 ± 1.49	Halliday et al. (1973b)
	50	6	5.13 ± 1.27	Halliday et al. (1973b)

b.w.: body weight; RBC: number of red blood cells; SD: standard deviation.

The overall confidence interval for the BMD_{05} was 0.10–4.88 mg/kg (see Figure I.5).

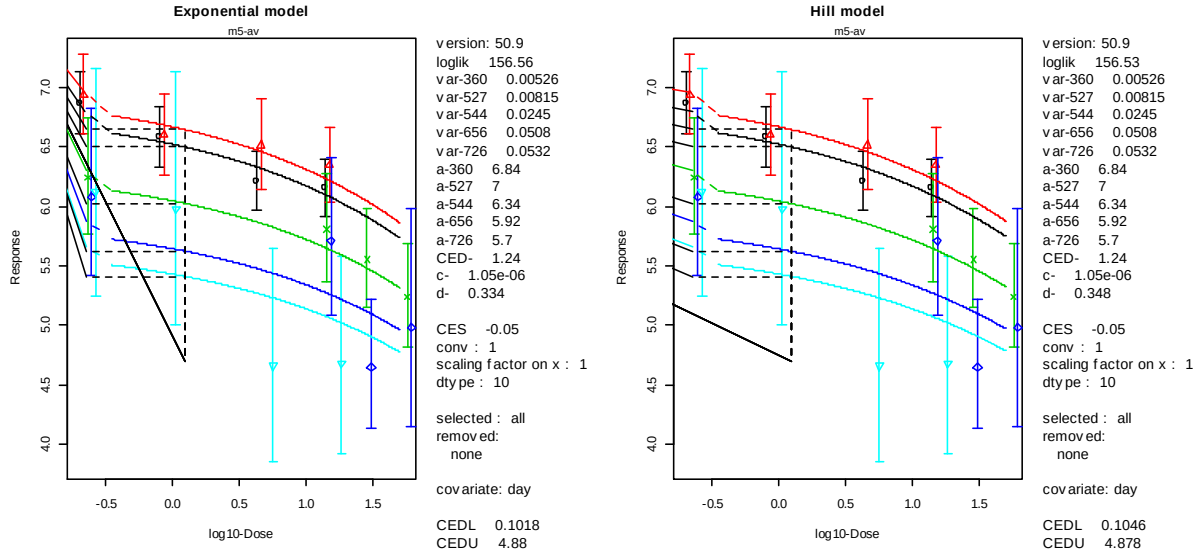


Figure I.5: Dose–response data and fitted model for red blood cells measured on various observation days (indicated by different marks and colours) in the two chronic studies on furazolidone. The background red blood cell levels for the various observation days are indicated in the right-hand side legend as parameter *a* followed by day number. The BMDs, BMDLs and BMDUs are shown in the legends on the right side of the figures (see CED, CEDL and CEDU). Note that the benchmark response is 5 %, indicated as CES (critical effect size) in the legend.

I .3. 3-amino-2-oxazolidinone: non-neoplastic lesions

Brinck et al. (1995) reported the effects of AOZ on four blood parameters (red blood cell count and levels of ALP, AST and bilirubin) in dogs (see Table I.11), all of which were subjected to a BMD analysis.

Table I.11: Dose–response data for the effect of 3-amino-2-oxazolidinone on four blood parameters in dogs as used for the benchmark dose analysis

Dose (mg/kg b.w. per day)	Number of animals	RBC ± SD (10^{12}/L)	ALP ± SD (µkat/L)	AST ± SD (µkat/L)	Biliburine ± SD (µmol/L)	Sex
0	3	5.59 ± 0.53	1.62 ± 0.19	0.46 ± 0.08	1.6 ± 0.1	M
1	3	5.23 ± 0.06	3.08 ± 0.39	0.57 ± 0.08	2.2 ± 0.26	M
3	3	5.08 ± 0.18	5.8 ± 0.51	0.88 ± 0.12	4.43 ± 1.15	M
6	3	4.59 ± 0.09	8.29 ± 4.56	1.35 ± 0.27	6.1 ± 0.44	M
0	3	6.37 ± 0.45	1.33 ± 0.11	0.6 ± 0.13	1.77 ± 0.15	F
1	3	5.64 ± 0.44	2.36 ± 0.41	0.64 ± 0.07	1.97 ± 0.21	F
3	3	5.07 ± 0.39	4.38 ± 0.59	0.81 ± 0.03	4.2 ± 0.79	F
6	3	4.82 ± 0.22	6.39 ± 0.7	1.17 ± 0.36	6.93 ± 2.69	F

ALP: alkaline phosphatase; b.w.: body weight; F: female; M: male; RBC: red blood cell count; SD: standard deviation.

I.3.1. Effect on red blood cell count in dogs (Brinck et al., 1995)

A significant difference in red blood cell count between males and females was found, but not in the sensitivity to AOZ. Therefore, the BMD_{05} confidence interval holds similarly for both sexes. The overall BMD_{05} confidence interval is 0.04–1.2 mg/kg b.w. per day (see Figure I.6).

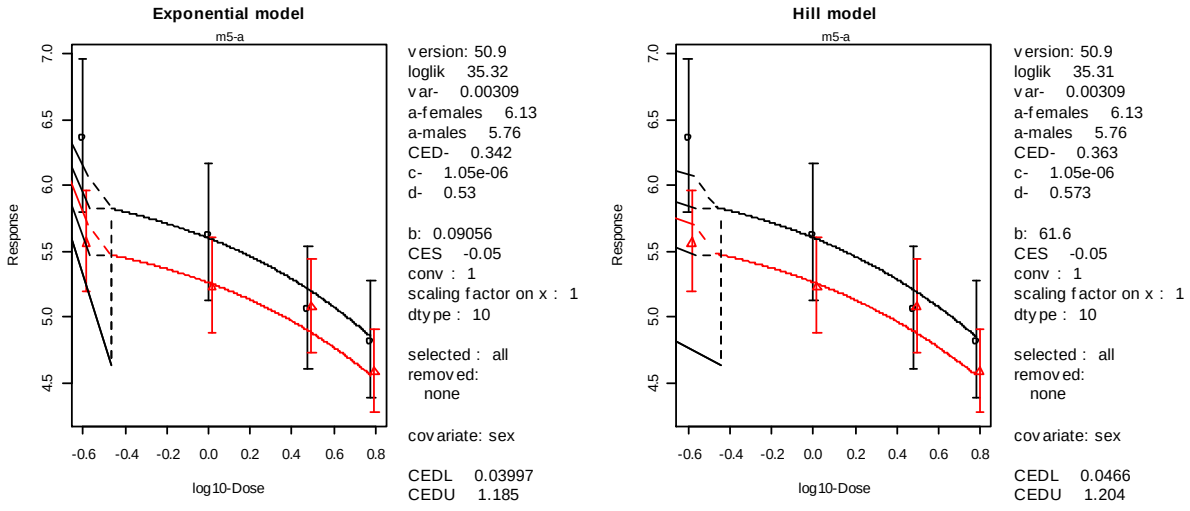


Figure I.6: Dose–response data for the effect of 3-amino-2-oxazolidinone (AOZ) on the number of red blood cells in dogs with fitted exponential and Hill model. The background red blood cell levels differ significantly among sexes, but not the sensitivity for AOZ. The BMDs, BMDLs and BMDUs are shown in the legends on the right side of the figures (see CED, CEDL and CEDU). Note that the benchmark response is 5 %, indicated as CES (critical effect size) in the legend.

I.3.2. Effect on serum alkaline phosphatase level in dogs (Brinck et al., 1995)

For ALP, both sexes differ significantly in sensitivity to AOZ. The overall BMD_{05} confidence interval in males is 0.02–0.18 mg/kg b.w. per day and in females 0.03–0.29 mg/kg b.w. per day (see Figure I.7, Table I.12).

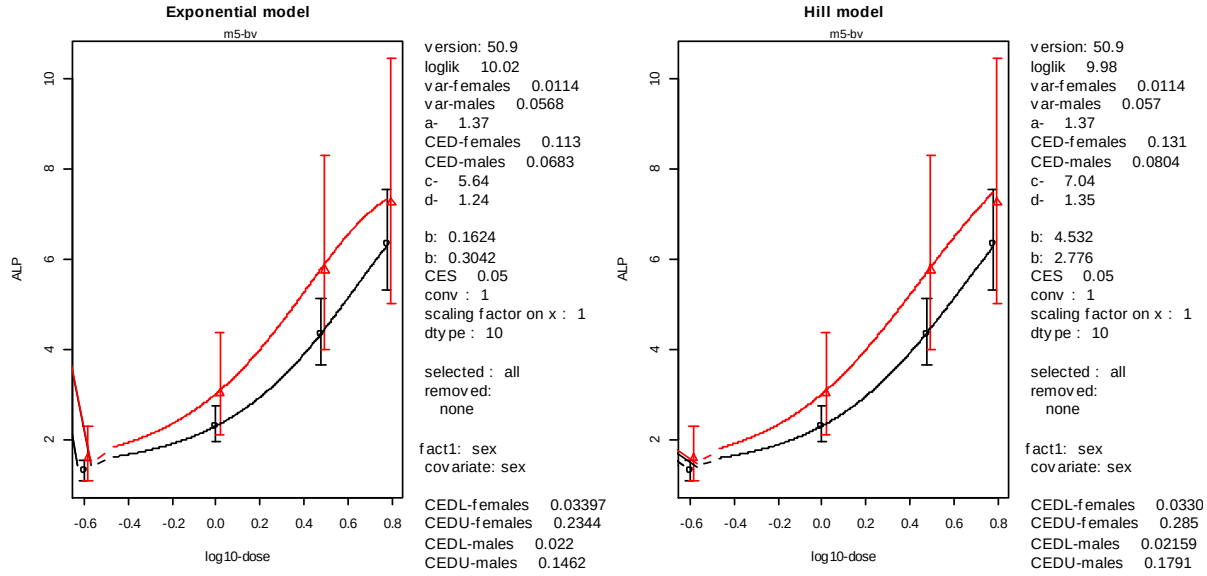


Figure I.7: Dose–response data for the effect of 3-amino-2-oxazolidinone (AOZ) on alkaline phosphatase levels in dogs with fitted exponential and Hill model. Sensitivity to AOZ differs significantly among sexes. The BMDs, BMDLs and BMDUs for males and females are shown in the legends on the right side of the figures (see CED, CEDL and CEDU). Note that the benchmark response is 5 %, indicated as CES (critical effect size) in the legend.

Table I.12: Benchmark dose results for the effect of 3-amino-2-oxazolidinone on alkaline phosphatase levels in dogs

Analysis with exponential models

Model	Converged	npar	Loglik
Full	1	9	7.76
Full-v	1	10	10.9
m1-v	1	3	–22.89
m2-v	1	4	–3.38
m2-av	1	5	–1.55
m2-bv	1	5	–2.67
m2-abv	1	6	–1.51
m3-v	1	5	5.41
m4-v	1	5	6.69
m5-v	1	6	6.93
m5-vab	1	8	10.66
m5-bv	1	7	10.02
m5-v	1	6	6.93
m5-bv	1	7	10.02

Analysis with Hill models

Model	Converged	npar	Loglik
full-v	1	10	10.9
m1-v	1	3	–22.89
m2-v	1	4	–8.76
m2-av	1	5	–7.54
m2-bv	1	5	–8.52
m2-abv	1	6	–7.49
m3-v	1	5	3.95
m4-v	1	5	6.66
m5-v	1	6	6.93
m5-vab	1	8	10.63
m5-bv	1	7	9.98
m5-v	1	6	6.93
m5-bv	1	7	9.98

I.3.3. Effect on serum aspartate aminotransferase level in dogs (Brinck et al., 1995)

For AST, males and females were found to differ significantly in sensitivity to AOZ, although the difference was not large. The overall BMD_{05} confidence interval for males is 0.08–0.9 mg/kg b.w. per day and 0.18–1.6 mg/kg b.w. per day for females (see Figure I.8).

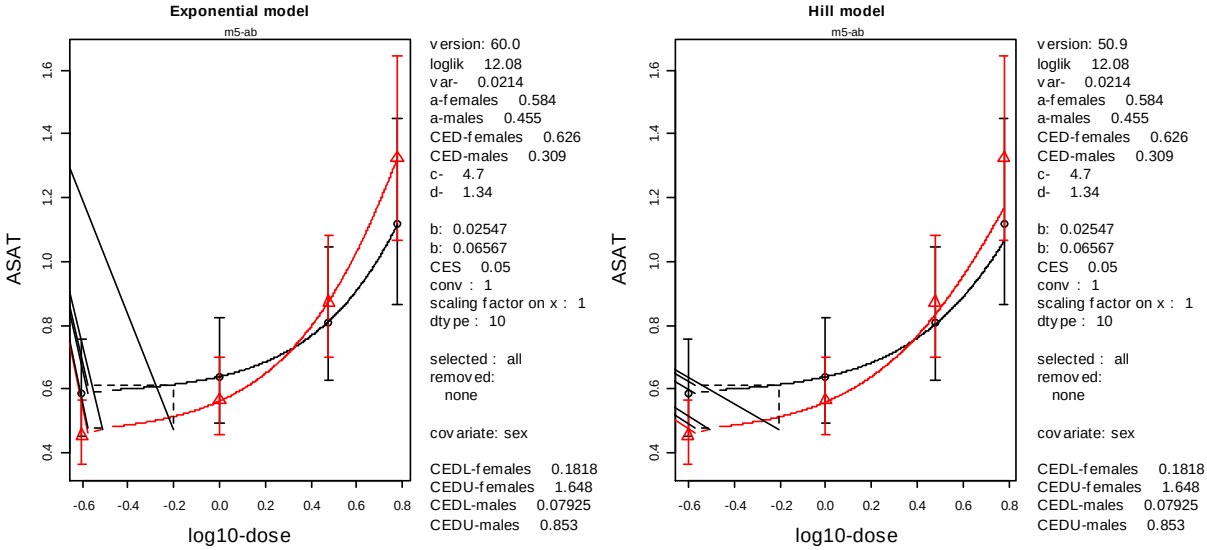


Figure I.8: Dose response data for the effect of 3-amino-2-oxazolidinone on aspartate aminotransferase in dogs with fitted exponential and Hill model. Males and females differed significantly in both a (background response) and b (sensitivity). The BMDs, BMDLs and BMDUs for males and females are shown in the legends on the right side of the figures (see CED, CEDL and CEDU). Note that the benchmark response is 5 %, indicated as CES (critical effect size) in the legend.

I.3.4. Effect on serum bilirubin levels in dogs (Brinck et al., 1995)

For bilirubin, males and females were not found to differ significantly in dose response. The overall BMD_{05} confidence interval is 0.20–0.97 mg/kg b.w. per day (Figure I.9).

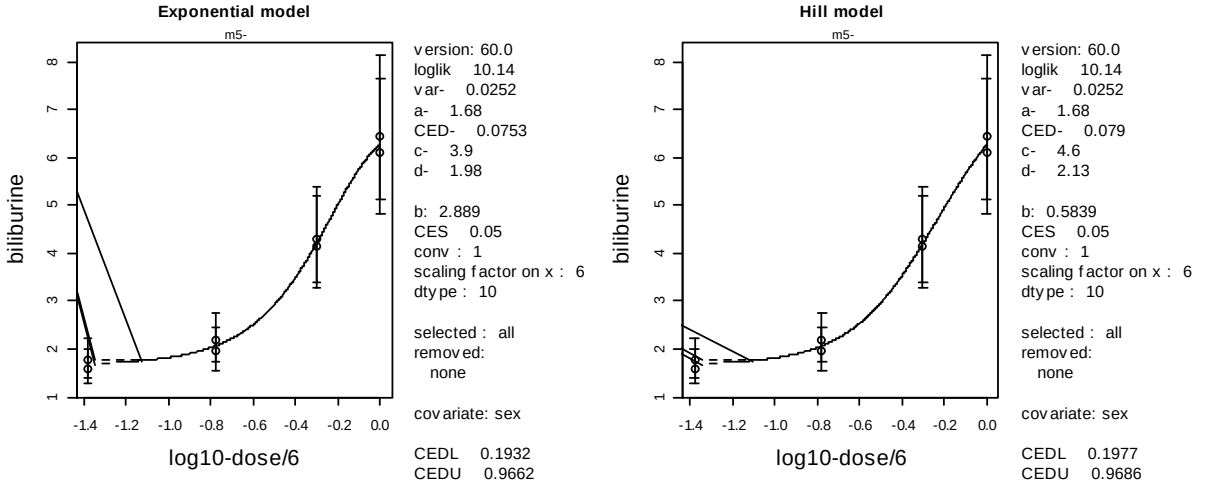


Figure I.9: Dose–response data for the effect of 3-amino-2-oxazolidinone on bilirubin in dogs with fitted exponential and Hill model. Males and females did not differ significantly in a (background response) or b (sensitivity). The BMDs, BMDLs and BMDUs for males and females are shown in the legends on the right side of the figures (see CED, CEDL and CEDU). Note that the benchmark response is 5 %, indicated as CES (critical effect size) in the legend. Regarding the CED note the scaling factor of 6.

I.3.5. Summary

Table I.13 shows the BMD results for the effect of AOZ on the four blood parameters considered. From these results, the CONTAM Panel selected the lowest $BMDL_{05}$ value of 0.02 mg/kg b.w. per day for the effect of AOZ on ALP.

Table I.13: Summary of benchmark dose results for non-neoplastic lesions caused by 3-amino-2-oxazolidinone in dogs

Target	Sex	$BMDL_{05}$ (mg/kg b.w. per day)	$BMDU_{05}$ (mg/kg b.w. per day)
Red blood cell count	M and F	0.04[(a)]	1.2
Serum alkaline phosphatase level	M	0.02	0.18
Serum alkaline phosphatase level	F	0.03	0.29
Serum aspartate aminotransferase level	M	0.08	0.9
Serum aspartate aminotransferase level	F	0.18	1.6
Serum bilirubin levels in dogs	M and F	0.2[(a)]	0.97

b.w.: body weight; $BMDL_{10}$: lower 95 % confidence limit for a benchmark response of 10 % extra risk; BMDU: benchmark dose upper confidence limit F: female M: male.
(a): Holds equally for each gender.

I.4. Furaltadone: mammary tumours

There is a chronic study on furaltadone (Cohen et al., 1973), but this study included only a single dose (other than the control). The average furaltadone dose was estimated to be 54 mg/kg b.w. per day, and a significant increase in the incidence of mammary adenocarcinoma (25/32 versus 0/25 in the control) was observed. Such data do not allow for a quantitative estimate of the potency, and therefore we combined the Cohen data with the data from the studies on furazolidone (see analysis above). In the analysis of the combined datasets, it was assumed that the shape parameter of the fitted model is the same among the two chemicals. This assumption is supported by the empirical finding that the shape parameter of fitted dose–response models in different carcinogens tends to be similar (Slob and Setzer, 2014). With this assumption, a finite BMD confidence interval may result, although the data for furaltadone include only one dose and a control.

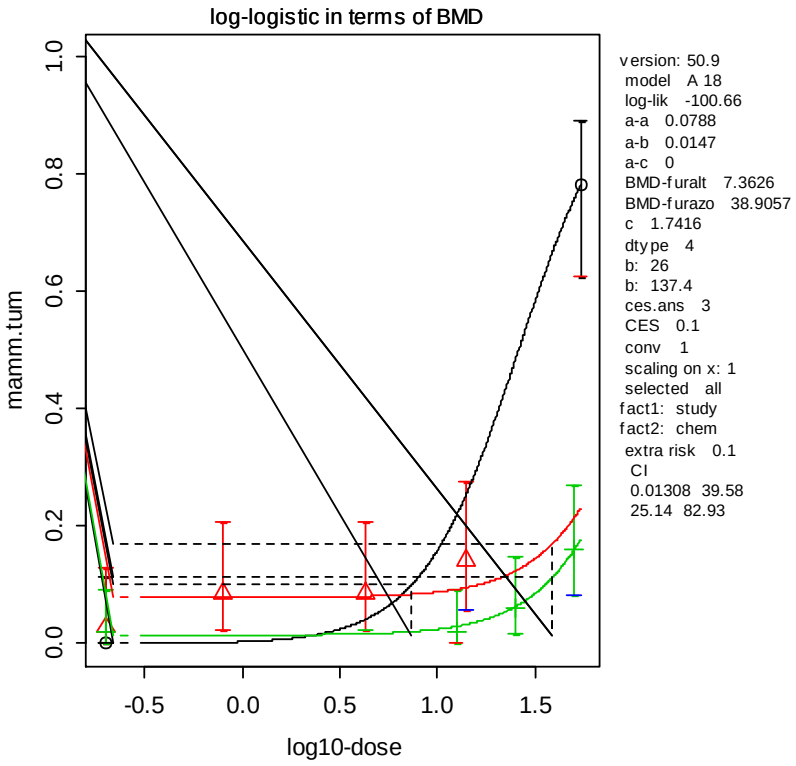


Figure I.10: The Cohen data for furaltadone (black circles) combined with the data for furazolidone (red triangles and green crosses). Note that this is only the best fit, and many more curves can describe these data (see Figure I.11).

In this analysis the background response (parameter a) is assumed to depend on study (hence three values), while the potency parameter b depends on compound (hence two values). The shape parameter c is assumed to be the same for the three datasets.

Figure I.11 shows the range of curves that are compatible with the combined dataset, assuming that the shape parameter is constant for all three subgroups. The black curves relate to the Cohen dataset. The wide variety of plausible curves results in a wide overall BMD_{10} confidence interval for furaltadone: 0.03–40 mg/kg. This large confidence interval is in this case because the observed response in the single dose in the Cohen study happens to be close to 100 %. Had the response been more intermediate, the confidence interval would have been smaller.

This analysis indicates that furaltadone is most probably more potent than furazolidone, as is also clear from just observing the confidence intervals around the observed responses (see Figure I.10). However, it is uncertain to what extent furaltadone is more potent.

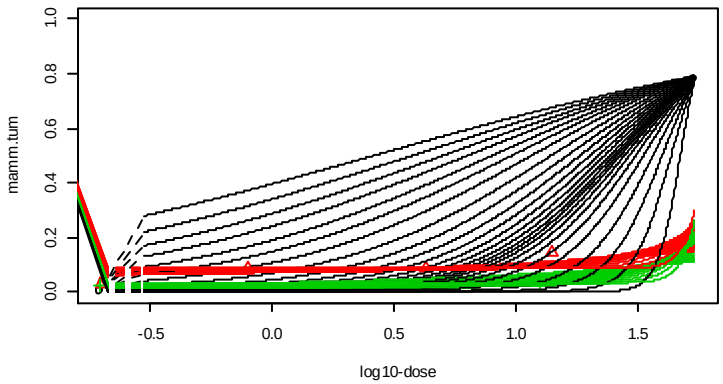


Figure I.11: A selection of curves that are compatible with the data for furaltadone (black curves) and for furazolidone (red and green curves), such that the shape parameter is the same for all three datasets

I.5. Nitrofurantoin: osteosarcomas

The chronic study with nitrofurantoin (NTP, 1989) resulted in few osteosarcomas in male rats, as shown in Table I.14. The results of the BMD analysis are summarised in Table I.15. The overall BMD_{10} confidence interval is 61 to infinite mg/kg b.w. per day. Thus, only a lower bound for the BMD_{10} could be assessed, since the BMD is not defined for a BMD confidence interval for which the upper bound is infinite. Due to the very low incidence of osteosarcomas, no graphical representation of the dose response data is presented.

Table I.14: Data on osteosarcomas in male rats for nitrofurantoin reported by NTP (1989) as used for the b enchmark dose analysis

Dose (mg/kg b.w. per day)	Number of rats	Number of rats with osteosarcomas
0	50	0
28	50	1
62	50	2

b.w.: body weight.

Table I.15: Benchmark dose[(a)] results for osteosarcomas caused by nitrofurantoin

Model	No.par	Log-likelihood	Accepted	$BMDL_{10}$ (mg/kg b.w. per day)	$BMDU_{10}$ (mg/kg b.w. per day)
Null	1	–14.71	–	NA	NA
Full	3	–13.3	(No)	NA	NA
Two.stage	3	–13.3	Yes	68.5	6 450
Log.logist	3	–13.3	Yes	61.9	Inf
Weibull	3	–13.3	Yes	61.7	Inf
Log.prob	3	–13.3	Yes	61.3	5.07×10^{8}
Gamma	3	–13.3	Yes	61.7	Inf
Logistic	2	–13.62	Yes	62.4	Inf

b.w.: body weight; $BMDL_{10}$: lower 95 % confidence limit for a benchmark response of 10 % extra risk; BMDU: benchmark dose upper confidence limit; Inf: infinity; NA: not available.
(a): No covariate; BMR: 0.1 extra risk; constraint: no; p-value goodness of fit: 0.05.

I.6. Nitrofurazone: non-neoplastic effects

For nitrofurazone, both a rat and a mouse study is available in which testes-related effects were found (Nishimura et al., 1995; George et al., 1996); see Table I.16 and I.17.

Table I.16: Dose–response data for the effect of nitrofurazone on testis and epididymis weight in rats (Nishimura et al., 1995)

Dose (mg/kg b.w. per day)	Number of rats	Testis weight ± SD	Epididymis weight ± SD
0	10	3.69 ± 0.34	1.2 ± 0.1
12.5	10	3.87 ± 0.22	1.18 ± 0.18
25	10	2.01 ± 0.42	0.84 ± 0.93
50	10	1.45 ± 0.17	0.74 ± 0.6

b.w.: body weight; SD: standard deviation.

Table I.17: Dose–response data for the effect of nitrofurazone on epididymal sperm number and testicular spermatid number in mice (George et al., 1996)

Dose (mg/kg b.w. per day)	Number of mice	Epididymal sperm number ± standard error of the mean	Testicular spermatid number ± standard error of the mean
0	40	933 ± 50	9.6 ± 0.4
14	20	817 ± 91	8.1 ± 0.4
51	20	716 ± 50	7.7 ± 0.4
102	20	15 ± 14	1.2 ± 0.3

b.w.: body weight.

I.6.1. Effect on testes weight

The overall BMD_{05} confidence interval for testis weight in rats is 12–19 mg/kg b.w. per day (Figure I.12).

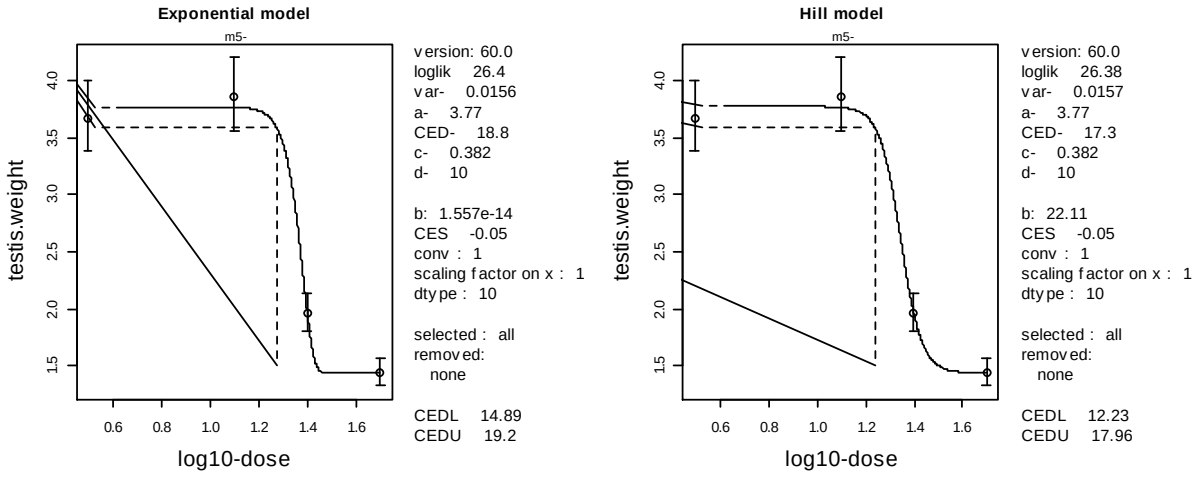


Figure I.12: Dose-response data for the effect of nitrofurazone on testis weights in rats with fitted exponential and Hill model. The BMDs, BMDLs and BMDUs for males and females are shown in the legends on the right side of the figures (see CED, CEDL and CEDU). Note that the benchmark response is 5 %, indicated as CES (critical effect size) in the legend.

I.6.2. Effect on epididymis weight

The overall BMD_{05} confidence interval for epididymis weight in rats is 4.6–20 mg/kg b.w. per day (Figure I.13).

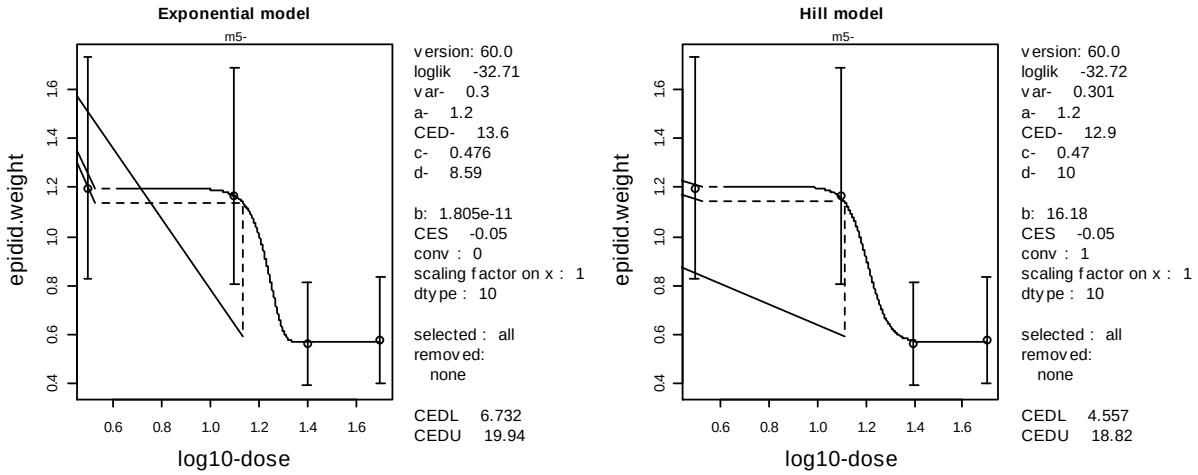


Figure I.13: Dose–response data for the effect of nitrofurazone on epididymis weights in rats with fitted exponential and Hill model. The BMDs, BMDLs and BMDUs for males and females are shown in the legends on the right side of the figures (see CED, CEDL and CEDU). Note that the benchmark response is 5 %, indicated as CES (critical effect size) in the legend.

I.6.3. Effect on epididymal sperm number

The overall BMD_{05} confidence interval for epididymal sperm number in mice is 25–92 mg/kg b.w. per day (Figure I.14).

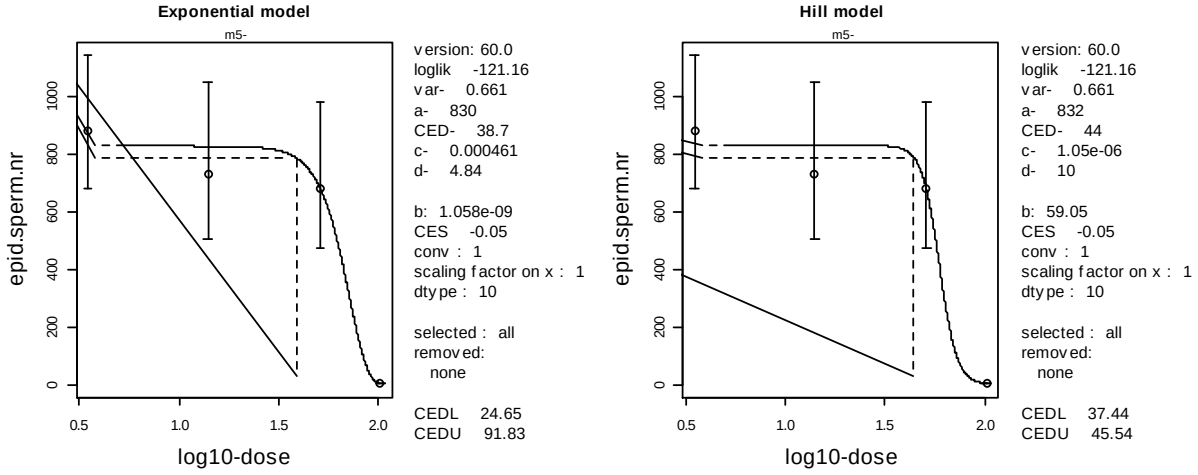


Figure I.14: Dose–response data for the effect of nitrofurazone on epididymal sperm number in mice with fitted exponential and Hill model. The BMDs, BMDLs and BMDUs for males and females

are shown in the legends on the right side of the figures (see CED, CEDL and CEDU). Note that the benchmark response is 5 %, indicated as CES (critical effect size) in the legend.

I.6.4. Effect on testicular spermatid number

The overall BMD_{05} confidence interval for testicular spermatid number in mice is 24–82 mg/kg b.w. per day (Figure I.15).

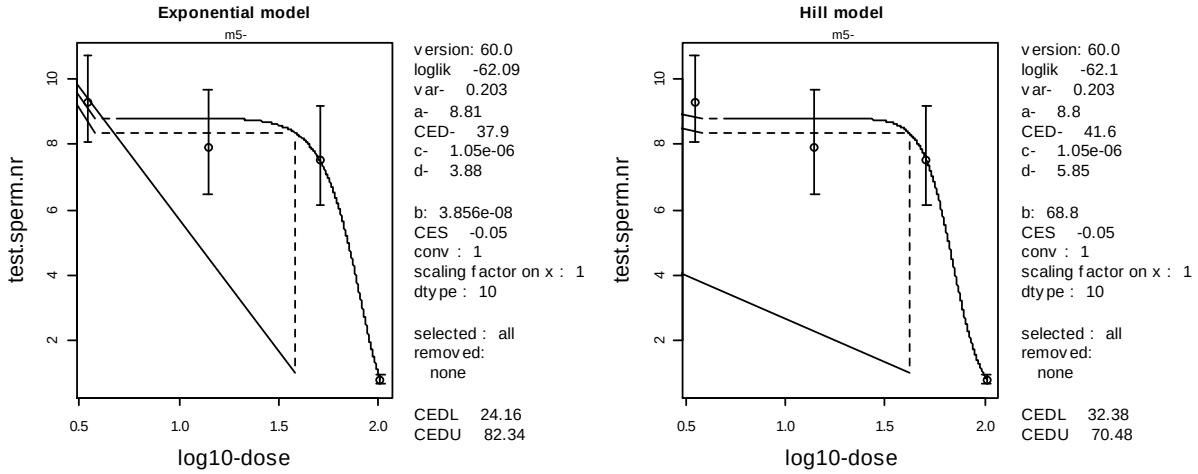


Figure I.15: Dose–response data for the effect of nitrofurazone on testicular spermatid number in mice with fitted exponential and Hill model. The BMDs, BMDLs and BMDUs for males and females are shown in the legends on the right side of the figures (see CED, CEDL and CEDU). Note that the benchmark response is 5 %, indicated as CES (critical effect size) in the legend.

I.6.5. Summary

Table I.18 shows the BMD results for the effect of nitrofurazone on the testis. From these results, the CONTAM Panel noted the lowest $BMDL_{05}$ value of 4.6 mg/kg b.w. per day for the effect of nitrofurazone on epididymis weight in rats.

Table I.18: Summary of benchmark dose results for non-neoplastic lesions caused by nitrofurazone

Target	Species	$BMDL_{05}$ (mg/kg b.w. per day)	$BMDU_{05}$ (mg/kg b.w. per day)
Testis weight	rat	12	19
Epididymis weight	rat	4.6	20
Epididymal sperm number	mice	25	92
Testicular spermatid number	mice	24	82

b.w.: body weight; $BMDL_{10}$: lower 95 % confidence limit for a benchmark response of 10 % extra risk; BMDU: benchmark dose upper confidence limit.

I.7. Semicarbazide: non-neoplastic effects

I.7.1. Disarrangement of epiphyseal chondrocytes tibia

Takahashi et al. (2014) reported the effects of semicarbazide on disarrangement of epiphyseal chondrocytes tibia in Wistar Hannover GALAS rats (see Table I.19). The results of the BMD analysis are shown in Table I.20. The overall BMD_{10} confidence interval is 1.0–3.8 mg/kg b.w. per day. Figure I.16 shows the data with the fitted log-logistic model.

Table I.19: Dose–response data for disarrangement of epiphyseal chondrocytes tibia in rats (Takahashi et al., 2014)

Dose (mg/kg b.w. per day)	Number of rats	Number of rats with disarrangement of epiphyseal chondrocytes tibia	Sex
0	10	0	M
0.6	10	0	M
3.5	10	5	M
16.7	10	10	M
0	10	0	F
0.8	10	0	F
4.5	10	2	F
21.8	10	10	F

b.w.: body weight; F: female; M: male.

Table I.20: Benchmark dose[(a)] results for disarrangement of epiphyseal chondrocytes tibia (semicarbazide)

Model	Par.covar	No.par	Log-likelihood	Accepted	$BMDL_{10}$ (mg/kg b.w. per day)	BMD_{10} (mg/kg b.w. per day)	$BMDU_{10}$ (mg/kg b.w. per day)	Sens.subgr
Null		1	–51.15	-				
Full		8	–12.36	-				
Two.stage	–	3	–14.35	Yes	1.55	2.05	2.81	–
Log.logist	–	3	–14.82	Yes	1.34	2.33	3.23	–
Weibull	–	3	–14.34	Yes	1.03	1.99	3.09	–
Log.prob	–	3	–14.52	Yes	1.31	2.28	3.19	–
Gamma	–	3	–14.33	Yes	1.09	2.11	3.12	–
Logistic	b	3	–11.94	Yes	1.61	3.17	3.78	m

b.w.: body weight; $BMDL_{10}$: lower 95 % confidence limit for a benchmark response of 10 % extra risk; BMDU: benchmark dose upper confidence limit.
(a): Covariate: sex; BMR: 0.1 extra risk; constraint: no; p-value goodness of fit: 0.05.

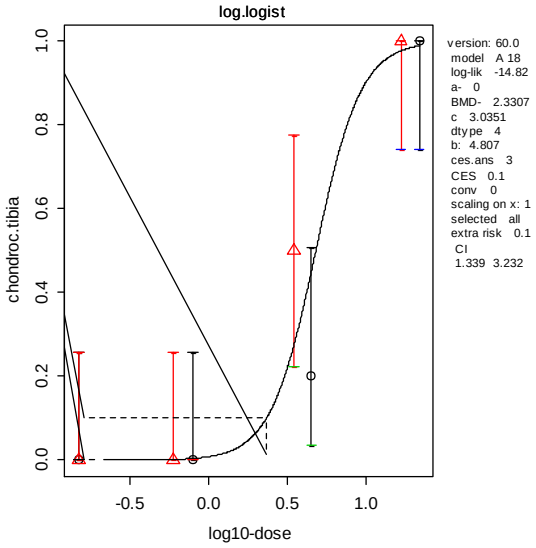


Figure I.16: Dose–response data for disarrangement of epiphyseal chondrocytes tibia with fitted log-logistic model. No significant differences in dose response were found between males (red triangles) and females (black circles)

I.7.2. Cleft palate in fetuses of treated Sprague–Dawley rats

Steffek et al. (1972) found effects from semicarbazide on cleft palate in Sprague–Dawley rat fetuses (see Table I.21). The results of the BMD analysis are shown in Table I.22, resulting in an overall BMD_{10} confidence interval of 5.4–21 mg/kg b.w. per day. Figure I.17 shows the data with the fitted log-logistic model.

Table I.21: Dose–response data for the effect of semicarbazide on cleft palate in Sprague–Dawley rats (Steffek et al., 1972)

Dose (mg/kg b.w. per day)	Number of fetuses	Number of fetuses with cleft palate
5	32	0
10	107	0
25	28	12
50	42	40
100	22	22

b.w.: body weight.

Table I.22: Benchmark dose [(a)] results for cleft palate in Sprague–Dawley rat fetuses (semicarbazide)

Model	No.par	Log-likelihood	Accepted	$BMDL_{10}$ (mg/kg b.w. per day)	$BMDU_{10}$ (mg/kg b.w. per day)
Null	1	–144.87			
Full	5	–27.16			
Two.stage	3	–38.21	No		
Log.logist	3	–27.83	Yes	5.4	20.9
Weibull	3	–30.3	No		
Log.prob	3	–27.44	Yes	14.8	20.6
Gamma	3	–28.06	Yes	14.9	20.5
Logistic	2	–31.61	No		

b.w.: body weight; $BMDL_{10}$: lower 95 % confidence limit for a benchmark response of 10 % extra risk; BMDU: benchmark dose upper confidence limit.
(a): No covariate; BMR: 0.1 extra risk; constraint: no; p-value goodness of fit: 0.05.

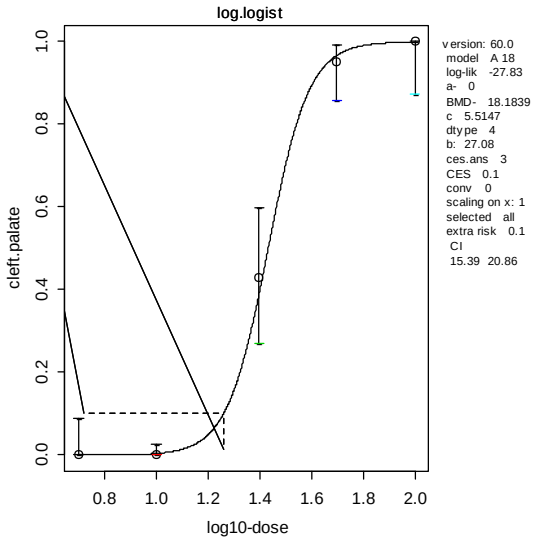


Figure I.17: Dose–response data for cleft palate in Sprague–Dawley rat fetuses with fitted log-logistic model

I.7.3. Summary

Table I.23 shows the BMD results for the effect of SEM on the non-neoplastic effects considered. From these results, the CONTAM Panel selected the lowest $BMDL_{10}$ value of 1.0 mg/kg b.w. per day for the effect of SEM on disarrangement of epiphyseal chondrocytes tibia.

Table I.23: Summary of benchmark dose results for non-neoplastic effects caused by semicarbazide in rats

Target	$BMDL_{10}$ (mg/kg b.w. per day)	$BMDU_{10}$ (mg/kg b.w. per day)
Disarrangement of epiphyseal chondrocytes tibia	1.0[(a)]	3.8
Cleft palate	5.4	21

b.w.: body weight; $BMDL_{10}$: lower 95 % confidence limit for a benchmark response of 10 % extra risk; BMDU: benchmark dose upper confidence limit.
(a): Holds equally for each gender.

I.8. Nifursol: non-neoplastic effects

I.8.1. Non-neoplastic effects reported by Wood et al. (1984)

Wood et al. (1984) reported the effects of nifursol on packed cell volume (PCV), mean corpuscular volume (MCV) and spleen weight in a 13-week study in rats (see Table I.24). The endpoints MCV and PCV in both sexes, and spleen weight in females were selected for BMD analysis.

Table I.24: Dose-response data for the effect of nifursol on packed cell volume, mean corpuscular volume and spleen weight in Sprague–Dawley rats (Wood et al., 1984)

Dose (mg/kg b.w. per day)	Number of animals	Sex	MCV[(a)] (fL)	PCV[(a)] (%)	Spleen weight (g)
0	10	M	68 ± 3.1	56 ± 1.9	n.m.
13.7	10	M	68 ± 2.6	55 ± 1.5	n.m.
28.0	10	M	67 ± 2.5	54 ± 1.2	n.m.
39.7	10	M	66 ± 3.4	55 ± 1.2	n.m.
53.6	10	M	63 ± 2.0	55 ± 2.5	n.m.
67.2	20	M	62 ± 2.5	53 ± 1.7	n.m.
0	10	F	70 ± 2.2	54 ± 1.8	0.53 ± 0.067
14.9	10	F	69 ± 2.3	53 ± 1.6	0.58 ± 0.067
30.2	10	F	69 ± 1.9	51 ± 2.0	0.62 ± 0.079
44.0	10	F	69 ± 4.0	50 ± 2.0	0.62 ± 0.079
61.8	10	F	67 ± 2.1	49 ± 2.8	0.59 ± 0.088
78.8	20 [(b)]	F	67 ± 2.4	49 ± 1.6	0.65 ± 0.097

b.w.: body weight; $BMDL_{10}$: lower 95 % confidence limit for a benchmark response of 10 % extra risk; BMDU: benchmark dose upper confidence limit; F: female; fL: femtolitre; M: male; MCV: mean corpuscular volume; n.m.: not modelled; PCV: packed cell volume.
(a): Data from week 13.
(b): For spleen weight only, 10 animals in high-dose group.

Males were found to be significantly more sensitive regarding MCV, while females were more sensitive regarding PCV (Figure I.18 and I.19).

The overall BMD_{05} confidence intervals are:

MCV: 28–47 mg/kg b.w. per day (males);

PCV: 18–39 mg/kg (females).

The dose–response analysis of spleen weight showed a significant trend, but the responses at all (five) non-zero doses were very similar (see Figure I.20), i.e. despite the large number of doses, there is very little dose–response information below the maximum response. As a result, the lower bound of the BMD is not determined by these data. It might also by hypothesised that the control group in this dataset was an outlier, while there is in reality no dose-related response. The latter is supported by another dataset on spleen weights in which the maximum response was found to be much larger.

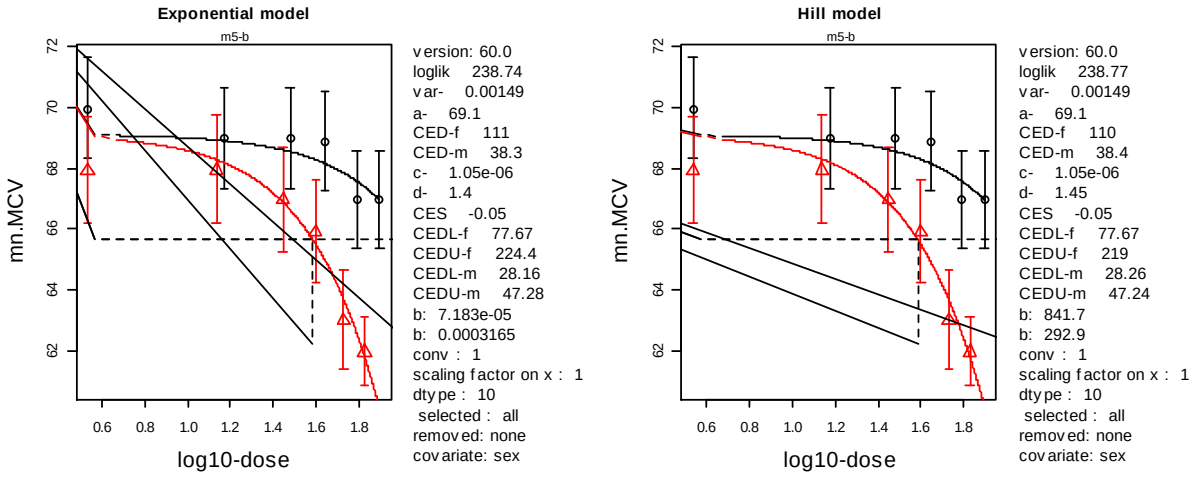


Figure I.18: Dose–response data for the effect of nifursol on mean corpuscular volume with fitted exponential and Hill model for males (triangles) and females (circles). The BMDs, BMDLs and BMDUs for males and females are shown in the legends on the right side of the figures (see CED, CEDL and CEDU). Note that the benchmark response is 5 %, indicated as CES (critical effect size) in the legend.

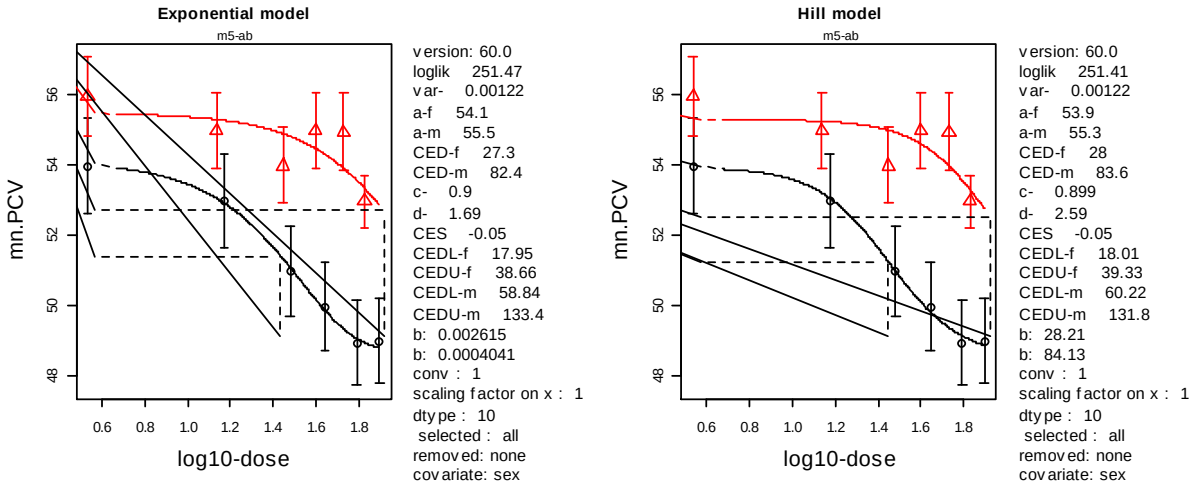


Figure I.19: Dose–response data for the effect of nifursol on packed cell volume with fitted exponential and Hill model for males (triangles) and females (circles). The BMDs, BMDLs and BMDUs for males and females are shown in the legends on the right side of the figures (see CED, CEDL and CEDU). Note that the benchmark response is 5 %, indicated as CES (critical effect size) in the legend.

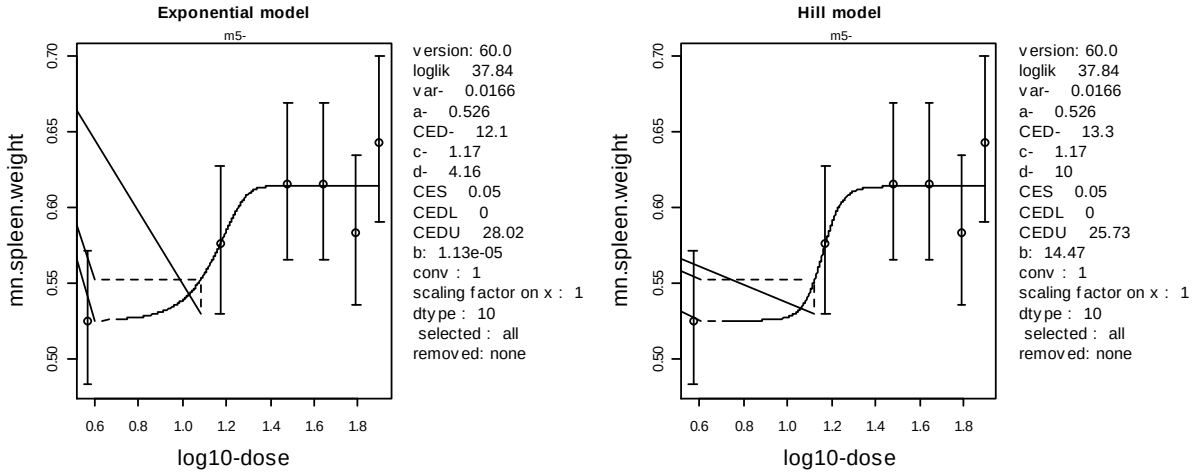


Figure I.20: Dose–response data for the effect of nifursol on spleen weights with fitted exponential and Hill model for females. The BMDs, BMDLs and BMDUs for males and females are shown in the legends on the right side of the figures (see CED, CEDL and CEDU). Note that the benchmark response is 5 %, indicated as CES (critical effect size) in the legend.

I.8.2. Effect of nifursol on liver weight (Rude et al., 1970c)

Liver weights in male Simonsen Long–Evans rats, reported by Rude et al. (1970c) showed a clear dose-related decrease in a 2-year rat study (Table I.25, with an overall BMD_{05} confidence interval of 11–26 mg/kg b.w. per day (Figure I.21, Table I.26).

Table I.25: Dose–response data for the effect of nifursol on liver weight in male Simonsen Long–Evans rats (Rude et al., 1970c)

Dose (mg/kg b.w. per day)	Number of animals	Liver weight ± SD (g)
0	37	15.1 ± 1.73
17.0	35	15.1 ± 1.9
25.9	36	13.3 ± 1.8
35.0	28	13.5 ± 1.7
43.5	39	12.3 ± 1.55

b.w.: body weight; SD: standard deviation.

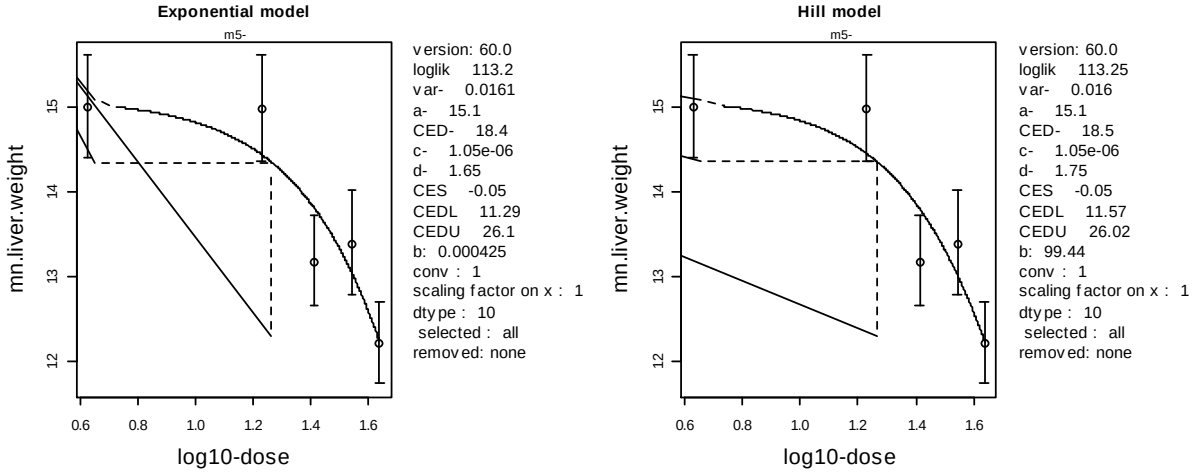


Figure I.21: Dose–response data for the effect of nifursol on liver weights with fitted exponential and Hill model for males. The BMDs, BMDLs and BMDUs for males and females are shown in the legends on the right side of the figures (see CED, CEDL and CEDU). Note that the benchmark response is 5 %, indicated as CES (critical effect size) in the legend.

Table I.26: Benchmark dose results for the effect of nifursol on liver weight in male rats

Analysis with exponential models

Model	Converged	npar	Loglik
Full	1	6	117.92
m1-	1	2	85.94
m2-	1	3	111.36
m3-	1	4	113.2
m4-	1	4	111.36
m5-	1	5	113.42

Analysis with Hill models

Model	Converged	npar	Loglik
Full	NA	6	117.92
m1-	1	2	85.94
m2-	1	3	110.87
m3-	1	4	113.25
m4-	1	4	110.87
m5-	1	5	113.8

I.8.3. Summary

Table I.27 shows the BMD results for the effect of nifursol on the non-neoplastic effects considered. From these results, the CONTAM Panel selected the lowest $BMDL_{05}$ value of 11 mg/kg b.w. per day for the effect of nifursol on liver weight.

Table I.27: Summary of benchmark dose results for non-neoplastic effects caused by nifursol in rats

Target	Sex	$BMDL_{05}$ (mg/kg b.w. per day)	$BMDU_{05}$ (mg/kg b.w. per day)
Mean corpuscular volume	M	28	47
	F	78	224
Packed cell volume	M	59	133
	F	18	39
Liver weight	M	11	26

b.w.: body weight; $BMDL_{05}$: lower 95% confidence limit for a benchmark response of 5 % extra risk; F: female; M: male.

ABBREVIATIONS

ABP	Androgen-binding protein
ADI	Acceptable daily intake
AFC Panel	EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
AHD	1-aminohydantoin
AIH	Autoimmune hepatitis
ALP	alkaline phosphatase
ALT	Alanine aminotransferase
AMOZ	3-amino-5-methylmorpholino-2-oxazolidinone
AOZ	3-amino-2-oxazolidinone
APCI	Atmospheric pressure chemical ionisation
AST	Aspartate aminotransferase
BCRP/ABCG2	Breast cancer resistance protein
BgVV	Federal Institute for Consumer Health Protection and Veterinary Medicine
b.i.d.	bis in die (twice daily)
BMD	Benchmark dose
BMDL	Benchmark dose lower confidence limit
$BMDL_{10}$	Lower 95 % confidence limit for a benchmark response of 10 % extra risk
BMDU	Benchmark dose upper confidence limit
BMR	Benchmark response
BrdU	Bromodeoxyuridine
b.w.	Body weight
CAS	Chemical Abstracts Service
CCα	Decision limit
CCβ	Detection capability
CD_{50}	Median convulsant dose
CED	Critical effect dose
CEDL	Critcal effect dose lower confidence limit
CEDU	Critcal effect dose upper confidence limit
CES	Critical effect size
CHL	Chinese hamster lung
CHO	Chinese hamster ovary
CI	Confidence interval
C_{max}	maximum serum concentration
CONTAM Panel	EFSA Scientific Panel on Contaminants in the Food Chain
CPAHD	1([(2-carboxyphenyl) methylene]-amine)-hydantoin
CPSEM	[(2-carboxyphenyl) methylene]-semicarbazide
CVMP	Committee for Veterinary Medicinal Products
CYP	Cytochrome P450
DAD	Diode-array detection
DMSO	Dimethyl sulphoxide
DNSH	3,5-dinitrosalicylic acid hydrazide
d.w.	Dry weight

EC	European Commission
EEA	European Economic Area (EEA
ELISA	Enzyme-linked immunosorbent assays
EMA	European Medicines Agency
EMEA	European Agency for the Evaluation of Medicinal Products
ESI	Electrospray ionisation
EU	European Union
F	Female
FAD	Flavine adenine dinucleotide
FAO	Food and Agriculture Organization
FAPAS	Food Analysis Performance Assessment Scheme
fL	Femtolitre
FL	Fluorescence detection
FMN	Flavine mononucleotide
FSA	Food Standards Agency
FSANZ	Food Standards Australia New Zealand
FSH	Follicle-stimulating hormone
G6PD	Glucose-6-phosphate dehydrogenase
GGT	Gamma-glutamyl transferase
GSH	Glutathione
GSSG	Glutathione-disulphide
HBGV	Health-based guidance value
HEH	2-hydroxy-ethylhydrazine
HPLC	High-performance liquid chromatography
IARC	International Agency for Research on Cancer
i.c.	Intracerebral
i.p.	Intraperitoneal
IRMM	Institute for Reference Materials and Measurements
i.v.	Intravenous
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC-MS	Liquid chromatography–mass spectrometry
LC-MS/MS	Liquid chromatography–tandem mass spectrometry
LD_{50}	Median lethal dose
LOAEL	Lowest-observed-adverse-effect level
LOD	Limit of detection
Log K_{ow}	Octanol/water partition coefficient
LOQ	Limit of quantification
M	Male
MAO	Monoamine oxidase
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
mm	Marker metabolite
MN	Micronucleus
MOE	Margin of exposure

MPL	Maximum permitted level
MRL	Maximum residue limit
MRPL	Minimum required performance limit
NADPH	Nicotinamide adenine dinucleotide phosphate
NA	Not applicable
NAT	N-acetyltransferases
NBA	2-nitrobenzaldehyde
ND	Not detectable
n.m.	Not modelled
NMC	Normochromatic micronucleated erythrocytes
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
NPAHD	1([(2-nitrophenyl) methylene]-amine)-hydantoin
NPAMOZ	3([(2-nitrophenyl) methylene]-amine)-5-methylmorpholino-2-oxazolidinone
NPAOZ	3([(2-nitrophenyl) methylene]-amine)-2-oxazolidinone
NPDNSH	3[(2-nitrophenyl) methylene]-5-dinitrosalicylic acid hydrazide
NPSEM	[(2-nitrophenyl) methylene]-semicarbazide
NR	nitroreductase
nr	Not reported
OTM	Olive tail moment
p.o.	per os (orally)
PCE	Polychromosome erythrocyte
PCV	Packed cell volume
PES	Processed *Euchema* seaweed
PMC	Polychromatic micronucleated
PND	Postnatal day
qs	*quantum satis*
RASFF	Rapid Alert System for Food and Feed
RIVM	National Institute for Public health and Environment (Rijksinstituut voor Volksgezondheid en Milieu)
ROS	Reactive oxygen species
RPA	Reference point for action
s.c.	subcutaneous
SCAN	Scientific Committee on Animal Nutrition
SCE	Sister chromatid exchange
SCO	Sertoli cell only
SEM	Semicarbazide
SPE	Solid phase extraction
TK	Thymidine kinase
T_{max}	Time to peak concentration
UDS	Unscheduled DNA synthesis
UHPLC	Ultra-high-performance liquid chromatography
UK	United Kingdom
USA	United States of America

USDA	United States Department of Agriculture
UV	Ultraviolet
VMP	Veterinary medicinal product
VSD	Virtual safe dose
WHO	World Health Organization