



FRONT OFFICE FOOD AND PRODUCT SAFETY

Assessment of toxicity of furazolidone and 3-amino-2-oxazolidinone (AOZ)

Risk assessment requested by: Netherlands Food and Consumer Product Safety Authority –
Bureau of Risk Assessment
Risk assessment performed by: RIVM
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Subject

Samples of soymix charges have been analysed for furazolidone by the Netherlands Food and Consumer Product Safety Authority. The soymix charges were delivered by an animal-feed trader to a compound feed manufacturer on March 15, 22 and 29 (2014). Subsequently the feed was provided to farmers whose cattle were subsequently tested positive for furazolidone. The three charges contained levels of furazolidone of respectively 25, 15.000 and 160 microgram/kg soymix (corresponding to 0,75, 450 and 4,8 microgram/kg feed). It is currently known that pig farmers were also provided with furazolidone-contaminated soymix.

Questions

- 1) Is it correct to state that the risk assessments of WHO (1993) and RIVM (2003) imply that a lifetime exposure of 3 microgram furazolidone per day would be associated with an acceptable risk?
- 2) Is it scientifically correct to assume that AOZ has the same carcinogenic potency as the mother compound furazolidone?

Conclusions

- 1) RIVM (2003) calculated a Virtual Safe Dose (VSD) for furazolidone of 50 ng/kg bodyweight/day based on data from WHO (1993), using linear extrapolation. This corresponds to 3 µg/day for a 60 kg person. As the risk at a VSD is considered acceptable, an exposure to furazolidone levels equal or lower than 3 µg/day VSD would be acceptable.
- 2) Based on data of a micronucleus test in mice which were administered AOZ, the carcinogenic potency of AOZ is predicted using a recently method developed by RIVM. Based on this prediction it may be concluded that the carcinogenic potency of AOZ is most probably lower than that of furazolidone.

Introduction

The toxicity of furazolidone has been assessed by JECFA in 1993 and EMA in 1997. More recently, studies on kinetics, metabolism and genotoxicity of furazolidone and/or its major metabolite 3-amino-2-oxazolidinone (AOZ) have been published. In 2003, RIVM performed a risk assessment for

consumption of shrimps containing AOZ. At that time, it was concluded that exposure to 50 ng furazolidone/kg bw/day (3 microgram furazolidone per day for a 60 kg person) during a lifetime would be associated with an extra cancer risk of 1 in a million.

Question 1

Is it correct to state that the risk assessments of WHO (1993) and RIVM (2003) imply that a lifetime exposure of 3 microgram furazolidone per day would be associated with an acceptable risk?

WHO (1993) did not estimate a 'Virtual Safe Dose'. WHO concluded that "because of the genotoxic and carcinogenic nature of furazolidone and deficiencies with respect to the data on the metabolites, the Committee was unable to establish an ADI".

Carcinogenicity studies using furazolidone have been performed in rats and mice. In rats, an increased tumour incidence (thyroid adenoma, mammary gland adenocarcinoma and basal cell epithelioma and carcinoma) was observed at doses of 25 and 50 mg/kg bw/day. In mice, tumours (bronchial adenocarcinoma, lymphosarcoma) were observed at doses of 12, 24 and 47 mg/kg bw/day (WHO, 1993).

RIVM (2003) calculated a Virtual Safe Dose (VSD) of 50 ng/kg body weight/day based on data from WHO (1993), using linear extrapolation. The VSD is the daily dose over a lifetime which is assumed to result in an extra cancer risk of 1 in a million; this risk (10^{-6}) is considered acceptable in the Netherlands (see http://www.rivm.nl/rvs/Normen/Consumenten/VSD_en_MTR). The VSD of 50 ng/kg bw/day corresponds to an exposure of 3 µg/day for a 60 kg person.

Note: the VSD is calculated for daily exposure over a lifetime. Obviously, for a very short exposure duration (one or several days) the same acceptable risk level will be achieved at much higher exposure levels (expressed per day) than the VSD.

Question 2

Is it scientifically correct to assume that AOZ has the same carcinogenic potency as the mother compound furazolidone?

Non carcinogenic effects

Furazolidone inhibits the enzyme monoamine oxidase (MAO) in mammals, an enzyme involved in the degradation of neurotransmitters in the body such as (nor)adrenaline, dopamine and serotonin. MAO-inhibition results in increased blood pressure and is probably the cause of the observed neurotoxicity (excitation) observed in laboratory animals (WHO, 1993). After repeated exposure, effects on liver (vacuolisation), decreased food consumption, decreased body weight and anaemia were observed in rats. Repeated exposure in dogs resulted in effects on the liver, anaemia, prolongation of thrombin time and cholestasis. Effects were already observed at doses of 1 mg/kg bw/day.

No toxicity- or toxicokinetic studies are available for metabolite AOZ, except for an in vitro MAO-inhibition study in pig hepatocytes (Hoogenboom et al., 1991, 2002) and an in vivo micronucleus test in mice (Hoogenboom et al., 2002; see below)

From the in vitro test it appeared that AOZ inhibits MAO. From the in vivo micronucleus test it can be concluded that mortality occurred in some animals when administered intraperitoneal doses of ≥ 125 mg AOZ/kg bw.

It is not clear whether the effects observed in the studies using furazolidone are caused by the mother compound and/or its metabolite AOZ or other metabolites. However, it can be concluded

that the above described effects occurred at doses much higher than the VSD of 50 ng/kg bw/day for carcinogenic effects of furazolidone.

Carcinogenic and genotoxic effects

Furazolidone is a mutagenic substance, causing DNA-damage in bacteria as well as mammalian cell systems. In bacteria, furazolidone was genotoxic with or without metabolic activation, which demonstrates that furazolidone itself is mutagenic. Furazolidone was demonstrated to be carcinogenic (see question 1) in both rats and mice. Based on the carcinogenicity data a VSD of 50 ng/kg bw/day was derived for furazolidone.

Almost no toxicity data are available for AOZ. Carcinogenicity of AOZ has not been directly assessed, though based on its genotoxic properties it may be expected that AOZ is involved in the carcinogenic properties of furazolidone. AOZ was found to be mutagenic in bacteria, with or without metabolic activation. AOZ causes chromosomal damage in in vitro human peripheral lymphocytes. This clastogenic effect is observed in the absence of metabolic activation. AOZ was demonstrated to cause DNA-damage in vivo as well: micronucleus tests in mice showed an increase in bone marrow micronuclei (Hoogenboom et al., 2002).

Insufficient data are available to make a direct comparison of the carcinogenic potency of furazolidone and AOZ.

RIVM recently developed a method to predict the carcinogenic potency of a substance based on the data of an in vivo micronucleus test in mice (Hernandez et al., 2012). This method is useful in the current assessment of the carcinogenic potency of AOZ which lacks any carcinogenicity data, while an in vivo micronucleus test is available. As just mentioned, Hoogenboom et al. (2002) measured the micronucleus incidence in mice bone marrow cells after administering AOZ in a single dose of 32, 62.5, 125, 250 and 500 mg/kg bw in males and 250, 500, 750, 1000, 1500 mg/kg bw in females (doses were based on two pilot studies). Bone marrow cells were sampled after 24 and 48 hour in five males and five females per dose and sampling time. In males, mortality was observed at a dose of 125 mg/kg bw (2 out of 10) and 500 mg/kg bw (2 out of 10); in females mortality was observed at a dose of 500 mg/kg bw (2 out of 10), 750 mg/kg bw (1 out of 10), 1000 mg/kg bw (2 out of 10) and 1500 mg/kg bw (1 out of 10). Hoogenboom et al. (2002) noted that, in view of the large difference in susceptibility to AOZ between males and females, the absence of a clear dose-response in toxicity and mutagenicity, and the interindividual variability in toxicity, mortality and mutagenicity, the result should be interpreted with care and they suggest that genotoxicity is exerted via a more than one step mechanism of action. However, based on the absence of further data it is concluded that AOZ is mutagenic in the mice micronucleus test.

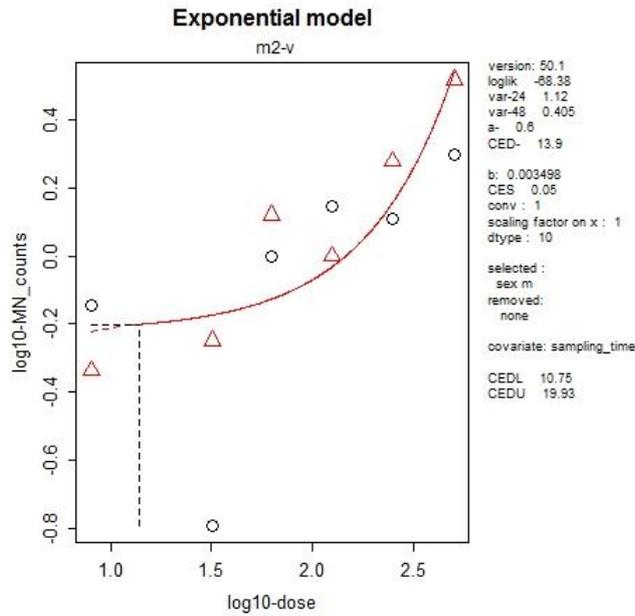


Figure 1. Effect of AOZ on micronucleus induction in bone marrow of male mice. Triangles and circles indicate the two sampling times.

The data in male mice as obtained in the micronucleus test of Hoogenboom et al. (2002) do show a clear dose response relation (see Fig. 1). Based on these data a $BMDL_{05}$ of 11 mg/kg bw was currently derived ($BMDU_{05} = 20$ mg/kg bw). In accordance with the RIVM method (Hernandez et al. 2012) the BMD_{10} for carcinogenicity would then be in the range of 11-2000 mg/kg bw/day. See Fig. 2 for illustration of this empirical relationship.

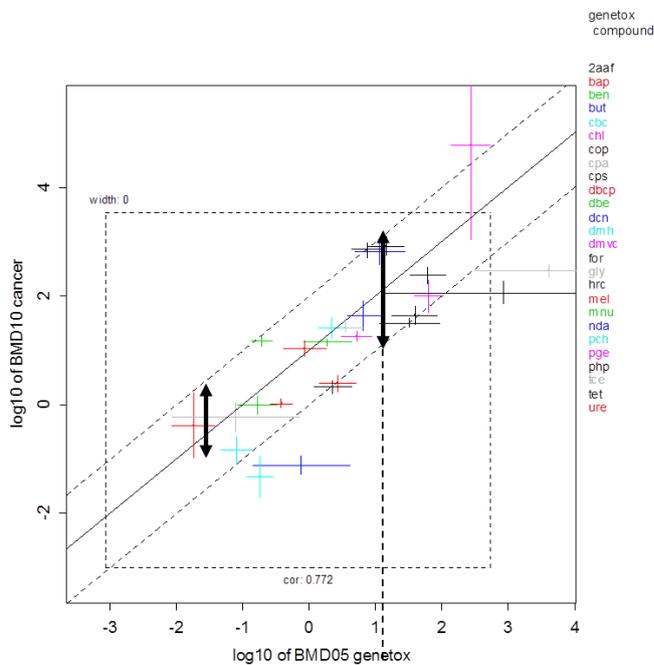


Figure 2. Tumour BMD_{10} plotted against (in vivo) micronucleus BMD_{05} for 26 substances. The 90%-confidence intervals are shown in both directions. The lower increasing dashed line reflects the

situation where the tumour BMD10 equals the micronucleus BMD05, the upper dashed line reflects the situation where the tumour BMD10 is a factor of 100 higher than the micronucleus BMD05. The smaller thick arrow reflects the uncertainty in the BMD10 when a carcinogenicity study is available (for a specific chemical), while the larger thick arrow reflects the uncertainty in the BMD10 when only the BMD05 from an in vivo micronucleus test is available (e.g. the vertical dashed line).

Based on the VSD of 50 ng/kg bw/day for furazolidone and the BMD₁₀ for AOZ a rough estimate can be made for the difference in carcinogenic potencies between furazolidone and AOZ. The VSD of 50 ng/kg bw/day (obtained via linear extrapolation to a 10⁻⁶ risk) corresponds to a BMD₁₀ (10⁻¹ risk) of about 5 mg/kg bw/day. This value for the BMD10 for furazolidone is lower than the uncertainty range of the BMD₁₀ of AOZ (i.e. 11 up to 2000 mg/kg bw/day). Therefore, it may be concluded that the carcinogenic potency of furazolidone is most probably higher than that of AOZ.

References

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