EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

Brussels, C7/GF/csteeop/Piperazine/hh/280504 D(04)

SCIENTIFIC COMMITTEE ON TOXICITY, ECOTOXICITY AND THE ENVIRONMENT (CSTEE)

Opinion on the results of the Risk Assessment of:

PIPERAZINE

Human Health part

CAS N°: 110-85-0

EINECS N°: 203-808-3

Carried out in the framework of Council Regulation (EEC) 793/93 on the evaluation and control of the risks of existing substances[1]

Adopted by the CSTEE during the 43rd plenary meeting of 28 May 2004

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[1] Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of those substances if they are produced or imported into the Community in volumes above 10 tonnes per year. The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC)1488/94, which is supported by a technical guidance document.

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Terms of Reference

In the context of Regulation 793/93 (Existing Substances Regulation), and on the basis of the examination of the Risk Assessment Report the CSTEE is invited to examine the following issues:

1. Does the CSTEE agree with the conclusions of the Risk Assessment Report

2. If the CSTEE disagrees with such conclusions, the CSTEE is invited to elaborate on the reasons for this divergence of opinion.

According to the Technical Guidance Document on Risk Assessment – European Communities 2003:

- *conclusion i): There is a need for further information and/or testing;*
- *conclusion ii): There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already;*
- *conclusion iii): There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.*

GENERAL COMMENTS

This is a well presented report and the CSTEE is able to support most of its conclusion. Piperazine was widely used as a drug for many years consequently there is reasonable data on its human effects following ingestion. Much less is known about the effects of oral and dermal exposure to piperazine. It is agreed that risks to the public from indirect exposure via the environment are minimal. The principal risks are to workers.

SPECIFIC COMMENTS

Exposure assessment

Piperazine base is strongly alkaline. It is used in the synthesis of various products including pharmaceuticals. For a number of years piperazine salts were used to treat intestinal worm infections. This used has largely ceased but piperazine salts are still used to treat intestinal worm infections in various domestic and farm animals.

In respect to the exposure two population groups may be identified:

a) Worker involved in the manufacture and use of piperazine and its salts

b) The general population

For both groups there are very few reliable measurements of actual exposure. The report has therefore had to resort to modelling data to estimate potential exposure. The approach used is a conservative one which is appropriate. It assumes for example that piperazine exposure either as the base or as any of the salts will result in 100% absorption by any exposure route. In the case of workers, the report concludes that the highest exposure is during the loading, final handling and during cleaning and maintenance. The conclusion is consistent with that for many other industrial chemicals. There is an apparent slightly inconsistency in assumptions made for situations in which ppe may be worn and those in which it is certain to be worn. Total exposure to piperazine is very hard to gauge due to the lack of measurements and the various situations in which piperazine exposure might occur. This includes exposure through consumption of food.

The report draws attention to the lack of reliable information on the metabolic fate of piperazine. To some extent the carbon skeleton of piperazine appears to be incorporated into the tissues indicating some initial breakdown to products of intermediary metabolism. However other unidentified metabolites are also produced. Particular research interest has centred on the ability of piperazine to combine with nitrite to form N-mononitrosopiperazine. This parallels the reaction of various other amines with nitrite. There is considerable controversy regarding the amount of this product formed.

Effects assessment

Acute effects

Piperazine has a low acute toxicity in animals and man. However there have been occasional reports of neurotoxicity when piperazine is administered to individuals with intestinal worm infections. It has been suggested that the mechanism of neurotoxicity involves GABA receptors antagonism.

Piperazine base is strongly alkaline and therefore is an irritant; however its salts have a much lower irritant potential.

Chronic effects

Neurotoxicity

Piperazine is a mild hepatotoxin and neurotoxin. In dogs which appear to be among the more sensitive laboratory animal NOEL values of 25 mg/kg body weight day^{-1} and 50 mg/kg body weight day^{-1} have been identified. Neurotoxic effects have also been observed in man generally at relatively high levels. However some individuals do appear to be rather more sensitive than the general population to the neurotoxic effects. Consequently a NOEL for humans has not been established satisfactorily. It is likely to be of the order of 30 mg/kg body weight day^{-1}

Reproductive effects

A number of developmental toxicity studies have been conducted in rats and rabbits. The effects have been rather non specific mainly a reduction in fecundity. There is no reliable data on reproductive effects in man.

Sensitization

Piperazine is a sensitizing agent in several animal models. In a study in mice using the lymph node essay a weak positive effect was observed. In a guinea pig maximization test some cross sensitization has been reported. A number of human case studies describe contact dermatitis. Piperazine has also been demonstrated to induce asthma in some workers. It must be concluded that piperazine is both dermal and respiratory sensitizing agent.

Genotoxicity

Piperazine and its phosphate, citrate, adipate, mebendazole and thiabendazole salts did not induce point mutations in bacterial tests. One poorly documented mouse lymphoma assay was positive in the presence of a metabolic activation system. Piperazine phosphate showed no activity with or without metabolic activation in one assay, and a weak activity in another study in the presence of S-9 mix. The latter, however, lacked reproducibility, and the increase in mutant frequency was still within the historical solvent control range. Piperazine phosphate did not induce chromosomal aberrations in Chinese hamster ovary cells.

In vivo, piperazine phosphate did not induce micronuclei in erythrocytes of CD-1 mice doses orally with 5,000 mg/kg body weight in a study which was performed in accordance with current standards. No indication of DNA damage was found in livers of partially hepatectomized Wistar rats dosed with 50 mg/kg body weight by single intraperitoneal injection, while in the same study single and double strand breaks were identified in animals dosed with 10 to 50 mg/kg body weight N,N'-dinitrosopiperazine. Piperazine was without effect in the host-mediated *S. typhimurium* mouse assay, while N-dinitrosopiperazine and N,N'-dinitrosopiperazine were tested positive in this assay. When piperazine dihydrochloride was co-administered with nitrite in equimolar doses to mice by gavage, the host-mediated assay was positive. From dose-response curve, the authors of the latter study (Braun et al., 1977) estimated a 50-70% nitrosation of piperazine dihydrochloride in the mouse stomach.

A significant increase in the frequency of micronuclei was found in cultured lymphocytes from 30 Swedish workers, exposed to a variety of chemicals, including piperazine and also genotoxic chemicals such as ethylene oxide. The increase was only evident if cell division was stimulated with pokeweed mitogen, but not if phytohemagglutinine was used. No increase in the frequency of micronuclei was found in the lymphocytes of 30 control subjects. Leukocytes from 76 exposed workers of the same cohort showed an increase in UDS activity (induced by N-acetoxy-N-acetyl-2-aminofluorene), and an increase in adenosine-diphosphate ribosyl transferase activity as compared to the controls. As the exposure may have involved over 100 chemicals, including well-established carcinogens, a causal relationship of these finding with piperazine exposure cannot be established.

Carcinogenicity

Human carcinogenicity data

An increase in cancer morbidity was observed for malignant lymphoma/myelomatosis in a retrospective cohort study including 664 male workers employed in a Swedish chemical plant. Because of co-exposure to a number of other chemicals, including carcinogens like ethylene oxide, epichlorohydrin, it is not possible to draw any valid conclusions from this observation. A case control study conducted within the cohort did not reveal any significant association with any specific chemical.

Animal carcinogenicity data

None of the available carcinogenicity studies with piperazine meets current standards.

Tumour induction was not observed in groups of 15 MRC rats per sex, administered 0.025% of piperazine with the drinking water for 75 weeks (ca. 20-25 mg/kg body weight day^{-1}). Piperazine did not induce a significant increase in the incidence of lung adenomas in groups of Swiss mice per sex, administered 6.3 g/kg with the diet (ca. 938 mg/kg body weight day^{-1}), for 28 weeks. In this study the only significant findings was a reduction in the number of malignant lymphomas in the piperazine treated animals. Feeding strain a mice with piperazine at 6.3 g/kg (ca. 938mg/kg body weight day^{-1}) or 18.8 g/kg (ca. 2,820mg/kg body weight day^{-1}) for 25 weeks, followed by a 13 weeks follow up post dosing, did not significantly increase the number of animals with lung adenomas.

N-mononitrosopiperazine and N,N'-dinitrosopiperazine were carcinogenic in rodents.

Risk characterization

For workers there is no concern in respect of acute toxicity. **Conclusion ii** is supported for skin irritation.

In regard to skin and respiratory sensitization the CSTEE agrees that **conclusion iii** is appropriate. The argument to support **conclusion iii** for reproductive effects is also acceptable. For neurotoxicity the case for the proposed **conclusion iii** is not strong and **conclusion i** is preferred.

Based on the available data on piperazine, there is little evidence for a relevant genotoxic effect. The CSTEE agrees with the conclusion that piperazine itself is not to be considered genotoxic. Its nitrosation products are however, *in vivo* mutagens.

The available data are not adequate to evaluate the carcinogenic risk. In view of the lack of a genotoxic activity, and the lack of any indication of a tumour promoting activity, it is unlikely that piperazine itself poses a relevant cancer risk. The two nitrosamine that can be formed from piperazine *in vivo* conditions (N-mononitrosopiperazine {NPZ} and N,N'-dinitrosopiperazine) induced mutation *in vivo*, and were carcinogenic in rodents, and it therefore appears that there is a certain cancer risk following exposure to piperazine. Unfortunately, the RAR does not include a quantitative estimation of this risk, and concludes "…*that there seems to be an additional cancer risk due to the formation of NPZ from piperazine, and although it is difficult to estimate, it is probably small*…" The information as presented in the RAR does not allow to judge whether the piperazine nitrosation rate may lead the relevant systemic nitrosamine exposure. There

are indications that the nitrosation rate might be substantial (50-70% as compared to 1-3% for morpholine in the same study) (Braun et al., 1977; study but not nitrosation rate is presented in the RAR). Information reported in the toxicokinetics section of the RAR also points to a possibly relevant nitrosamine formation in man; however, due to the limited data, it is not possible to judge whether this relevant for the cancer risk.

Based on the present data, a relevant contribution of nitrosamines to the cancer risk cannot therefore be excluded. The CSTEE recommends to change **conclusion ii** into **conclusion i** and to generate appropriate *in vitro* and/or *in vivo* data on the extent of endogenous nitrosamine formation, and the accompanying cancer risk.

References

Braun R., Schoeneich J. and Ziebarth D. (1977) In vivo formation of N-nitroso compounds and detection of their mutagenic activity in the host-mediated assay. Cancer Research 37(12), 4572-4579.