EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Health and Environmental Risks

SCHER

Risk Assessment Report on 2-nitrotoluene

Human Health Part

CAS No.: 88-72-2

EINECS No.: 201-853-3

Scientific Committees
on consumer products
on emerging and newly identified health risks
on health and environmental risks

The SCHER adopted this opinion at its 21st plenary on 15 January 2008

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCHER

Questions relating to examinations of the toxicity and ecotoxicity of chemicals, biochemicals and biological compounds whose use may have harmful consequences for human health and the environment.

In particular, the Committee addresses questions related to new and existing chemicals, the restriction and marketing of dangerous substances, biocides, waste, environmental contaminants, plastic and other materials used for water pipe work (e.g. new organics substances), drinking water, indoor and ambient air quality. It addresses questions relating to human exposure to mixtures of chemicals, sensitisation and identification of endocrine disrupters.

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http://ec.europa.eu/health/ph_risk/risk_en.htm

ACKNOWLEDGMENTS

The rapporteur is acknowledged for her valuable contribution to this opinion:

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Keywords: SCHER, scientific opinion, risk assessment, Regulation 793/93, human health, CAS 88-72-2, 2-nitrotoluene

Opinion to be cited as:

SCHER, scientific opinion on the risk assessment report on 2-nitrotoluene, human health part, CAS 88-72-2, 15 January 2008

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1. BACKGROUND

Council Regulation 793/93 provides the framework for the evaluation and control of the risk of existing substances. Member States prepare Risk Assessment Reports on priority substances. The reports are then examined by the Technical Committee under the regulation and, when appropriate, the Commission invites the Scientific Committee on Health and Environmental Risks (SCHER) to give its opinion.

2. TERMS OF REFERENCE

On the basis of the examination of the Risk Assessment Report the SCHER is invited to examine the following issues:

1) Does the SCHER agree with the conclusions of the Risk Assessment Report?
2) If the SCHER disagrees with such conclusions, it is invited to elaborate on the reasons.
3) If the SCHER disagrees with the approaches or methods used to assess the risks, it is invited to suggest possible alternatives

3. OPINION

3.1. General comments

The document is written in accordance with the requirement of the TGD, and all relevant endpoints are addressed, with a major focus on mutagenicity and genotoxicity. The RAR is somehow uncritical and report many studies that do not meet OECD guidelines or GLP standards. In addition, the sections on mutagenicity and metabolism are more of academic interest, than being useful for the risk assessment. The summary sections should be concise and only summarize information already presented, and not introduce new data. SCHER recommends that the text of the document only report on acceptable and relevant studies to be used in the risk assessment.

The information on production and sites are generally based upon older data, and manufacturing has stopped at many sites, e.g. UK.

3.2. Specific comments

3.2.1. Exposure assessment

Only inhalation and dermal exposure are considered relevant for the occupational settings, whereas consumer exposure does not occur. Indirect environmental exposure is based upon 3 different scenarios and a regional site.

The occupational exposure is only based upon one location, production and processing of 2-NT, and it is assumed to be the Italian manufacturer.

Occupational exposure, both inhalation and dermal, is based upon the EASE model, rather than using measured values for inhalation exposure. The highest measured value reported was 0.280 mg/m^3, whereas the values used in EASE model are 0.35-0.7 mg/m^3. An absorption rate of 100% was used in the assessment. This value is not based upon experimental studies, and is considered very conservative, e.g. an absorption rate of only 10% was used for similar compound, 2,4-dinitrotoluene. A RWC of 420 mg/day is a very high estimate knowing that dermal exposure is mostly incidental, and that PPE will be applied. PPE has not been taken into consideration.

Human exposure via the environment was estimated using the EUSES model at the three major sites for production. It should be noted that production no longer occurs at the UK sites, only processing.

3.2.2. Effect assessment

This section covers all the relevant endpoints as required by the TGD. However, all the animal studies are based upon route of exposure that is not relevant for human exposure.

The effect assessment section includes a very detailed description of the ADME studies, many of which are only of academic interest. This part is very extensive and complex and the metabolic chart should have been presented early to facility the reading. Furthermore irrelevant data has been included, e.g. hippuric acid excretion, a metabolite of toluene. No data on metabolism in humans have been reported, but recent studies in workers occupationally exposed to high level of 2-NT indicate that 2-nitrobenzylalcohol is the major urinary metabolite (Sabbioni,2006). Studies in germ-free animal vs. conventional animals indicate that the active mutagenic metabolites is formed by bacterial transformation of 2-NT to o-toluidine. The requirement for bacterial biotransformation could also explain the lack of *in vitro* mutagenicity. O-toluidine is a strong in vitro mutagen (IARC,2000).

Mutagenicity

Both *in vitro* and *in vivo* mutagenicity studies were conducted. Bacterial mutagenicity studies using S.*typhimirium* with and without S9 were all negative. But the strain used did not express nitroreductase activity. A recent study using nitroreductase proficient S. *typhimurium* strains showed the activation of 2-NT to a mutagenic metabolite (Salamanca-Pinzón, 2006). I*n vitro* mutagenicity studies in mammalian cells were generally negative, however a slight increase in sister chromatid exchange was observed in Chinese hamster ovary cells. 2-NT was also generally negative in *vivo* bone marrow micronucleus test in mouse and rats, however a slight increased frequency was observed in male rats at the highest dose. Covalent binding of 2-NP to hepatic DNA has been reported in male rats following oral exposure.

Carcinogenicity

Several types of cancers have been reported in guideline quality studies, mesothelioma in rat being the most sensitive malignant tumour endpoint following oral exposure. No studies using the relevant exposure routes, inhalation and dermal absorption, are reported.

A recent study also demonstrated that the mesothelioma in rats exposed to 2-NT was similar at the cellular and molecular level to mesothelioma in humans (Kim, 2006), making this tumour type relevant for risk characterisation Newer studies show that 2-NT induces large intestine tumours mice, and that the molecular pathology is similar to the one seen in man, i.e. activation of cancer relevant genes (Sills, 2004). The sex differences in carcinogenicity observed in rats was explained by the greater biliary excretion in males than females, however the incidence of mesothelioma was higher in male rats fed 2-NT than o-toluidine, the mutagenic metabolite formed by bacterial conversion (NTP, 2000).

Humans

There is limited data on the effect on humans and they have been presented in a Hazardous Substance Bank (2004) that are not available for review and mostly based upon old case reports and no concentrations have been given for the various effects.

3.2.3. Risk characterisation

The risk characterization performed in the RAR using the margin-of safety (MOS) approach for non-cancer endpoints and is performed for inhalation and dermal exposures. TD25 is used to assess the cancer risk.

The SCHEER agrees on the use of the Margin of Safety (MOS) approach to evaluate the non-cancer toxicities, using a LOAEL from the chronic oral carcinogenicity study, and the default value on 100% uptake by the inhalation and dermal routes of exposure.

Workers

The TD25 approach has been used to asses the MOS for carcinogenicity using data on combined fibroma and fibrosarcoma group. The SCHER disagree that the concurrence of fibroma and fibrosarcoma in the same animal is proof that the fibroma is a precursor for the fibrosarcoma. Thus using the HT25 for the combined tumour group for determination of the life-time cancer risk is not relevant, and SCHER recommends that the HT25 for mesothelioma should be used in the risk characterization.

Although the exposure estimate is very conservative, SCHER agrees with conclusion (iii)[1] for carcinogenicity

The SCHER also supports the conclusion (ii) regarding reproductive and developmental toxicity following inhalation, but does not support the conclusion (iii) following dermal exposure due to the large uncertainty in determination of exposure using the EASE modelling.

The SCHER agrees with the conclusion (i) on hold that has been proposed for skin sensitisation, as 2-NT is a genotoxic carcinogen.

Consumers

2-NT has not been detected in consumer products, and thus there is no anticipated consumer exposure for 2-NT, and SCHER recommend the conclusion (ii).

Man exposed via the environment

The risk was characterized at the 3 different locations for production and processing and at a regional site. The SCHER supports the conclusion (ii) for chronic, reproductive and developmental toxicity. However, the SCHER disagree with conclusion (iii) for mutagenicity and carcinogenicity. *In vivo* mutagenicity was only observed at concentrations significantly higher than the relevant exposure even at site C. For carcinogenicity, the estimate was based upon the combined fibroma/fibrosarcoma group, and only at site C the tolerable risk for humans exposed via the environment was exceeded. Using the HT-25 for mesothelioma, the tolerable risk at site C was not exceeded.

4. LIST OF ABBREVIATIONS

ADME	Absorption, Distribution, Metabolism, Excretion
EASE	Estimation and Assessment of Substance Exposure
EUSES	EU System for the Evaluation of Substances
GLP	Good laboratory practice
LOAEL	Lowest Observable Adverse Effect Level
MOS	Margin of Safety
2-NT	2-nitrotoluene
PPE	Personal Protective Equipment

[1] *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

RAR	Risk Assessment Report
RWC	Reasonable Worst Case
TD25	Dose at which 25% of the treated animals develop tumours
TGD	Technical Guidance Document

5. REFERENCES

Creely KS, Tickner J, Soutar AJ et al (2005) Evaluation and further development of EASE model 2.0 Ann Occup Hyg 49: 135-145.

IARC (2000) IARC monographs on the evaluation of carcinogenic risks to humans Vol 77, 267

Kim Y, Ton TV, DeAngelo AB et al (2006) Major carcinogenic pathways identified by gene expression analysis of peritoneal mesotheliomas following chemical treatment in F344 rats. Toxicol Appl Pharmacol 214: 144-151.

NTP(2000) NTP Comparative toxicity and carcinogenicity studies of o-nitrotoulene and o-toulidine hydrochloride (CAS Nos 88-72-2 and 636-21-5) administered in feed to male F344/N rats. Toxic Rep Ser 44:1-C8.

Sabbioni G, Jones CR, Sepai O et al (2006) Biomarkers of exposure, effect, and susceptibility in workers exposed to nitrotoluenes. Cancer Epi Biomarker Preven 15: 559-566.

Salamanca-Pinzón SG, Camacho-Carranza R et al (2006) Nitrocompound activation by cell-free extracts of nitroreductase-proficient Salmonella typhimurium strains. Mutagenesis 21: 369-374

Sills RC, Hong HL, Flake G et al (2004) O-nitrotoulene-induced large intestine tumours in B6C3F1 mice model human colon cancer in their molecular pathogenesis. Carcinogenesis 25: 605-612.