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

Opinion on the results of the Risk Assessment of:

Aniline

Human Health part

CAS N° : 62-53-3

EINECS N° : 200-539-3

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 37th plenary meeting of 1 April 2003

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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.

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

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Aniline

Human Health Effects

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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.

INTRODUCTION

Aniline is exclusively used as a chemical intermediate, with 71% being processed to 4,4'-methylenedianiline (MDA), the starting material for polyurethane plastics. Other industrial uses are for processing to caoutchouc chemicals (15%), dyes (5%), pesticides (3%), pharmaceuticals (1.2%), fibres (1%) and others (3.7%). Aniline is being produced or imported by 9 European companies, a projected production volume for 1998 was 605,000 tonnes/year, whereas 50,000 tonnes/year was estimated to be imported in 1998. However, the actual production and use of aniline has increased more than this prediction, especially the amount of aniline processed to MDA, but exact figures are lacking.

GENERAL COMMENTS

The human health part of the RAR is of very good quality. The CSTEE agrees with the conclusion iii) for workers due to the carcinogenic effects of aniline in combination with the indications of genotoxic properties. Specific high risk scenarios resulting from inhalation of aniline were identified during production and further processing in the large-scale chemical industry and in certain other working areas. These situations give

concern for chronic toxicity as well. In addition, some scenarios also give rise to concern regarding acute toxicity. Without proper protection, dermal occupational exposure to aniline will give rise to concern regarding carcinogenicity and chronic toxicity. Furthermore, aniline is a skin sensitiser. The CSTEE agrees with the RAR conclusions that risk reduction measures have to be initiated and that occupational exposure limits should be reconsidered.

SPECIFIC COMMENTS

HUMAN HEALTH RISK ASSESSMENT

1. Exposure assessment

Inhalation and dermal exposure to aniline during production ad further processing are the main exposure scenarios. In addition, exposures are to be expected if formulations with residual aniline contents are handled, such as dyes and adhesives, or if aniline occurs during further processing as a result of decomposition, such as in foundries and during rubber vulcanisation. The RAR has estimated the internal body burden of workers from inhalation and dermal exposure for the various exposure scenarios, based on measured and modelled data. The highest body burdens were found for production of aniline and further processing in the large-scale chemical industry. High exposures were also determined for release of aniline as a decomposition product in iron, steel and aluminium foundries and use of liquid dyeing formulations with residual aniline.

2. Effects assessment

Toxicokinetics

Aniline is well absorbed after oral and inhalation exposure, approximately 90% of an oral dose is absorbed in rats. Dermal absorption in humans was estimated to amount up to 38%. The major contributors to aniline clearance appear to be a combination of acetylation and hydroxylation reactions. The glucuronide and sulphate conjugates of 4-hydroxyacetanilide represent the major urinary metabolites of aniline. N-Hydroxylation of aniline to N-phenylhydroxylamine is the principal route by which aniline produces toxic effects, including methaemoglobinaemia. It is well known that N-acetylation reactions are genetically polymorphic in nature.

Acute toxicity

Acute intoxication of humans with aniline has been reported frequently in the past. The average lethal inhalation dose for humans is reported to be 25 mg/l air or 0.35-1.43 g/kg bodyweight. With respect to methaemoglobin formation the no-effect dose of aniline in adult human is about 0.2 mg/kg bodyweight. In animal experiments the acute toxicity of aniline shows significant species differences, with cats being much more sensitive than conventional laboratory animals.

Irritation/corrosion

Aniline is a weak irritant to the skin of rabbits, no data on skin irritation in humans are available. Aniline is a strong irritant when tested in rabbit eyes.

Sensitisation

In humans, allergic skin reactions to aniline have been reported, mainly in patients suffering from eczematous dermatitis. The positive reactions were often associated with para-group cross reactivity. In animals, aniline revealed a mild to moderate sensitisation potential. Respiratory sensitisation has not been observed, but due to the observed skin sensitisation, it cannot be ruled out.

Repeated-dose toxicity

The main endpoint of aniline after repeated dosing, irrespective of route of administration, is toxicity to the haematopoietic system with erythrotoxicity, methaemoglobinaemia, haemolytic anaemia and formation of Heinz bodies, with corresponding changes of the spleen, the bone marrow, the kidneys and the liver. The damaged red blood cells are scavenged predominantly in the red pulp of the spleen, followed by increased haemosiderin accumulation. Multifocal perisplenitis was seen after 4 weeks of oral administration in rats. After chronic administration also stromal hyperplasia and fibrosis, and chronic capsulitis occurred in the spleen.

A general problem with the repeated-dose toxicity studies with aniline is that a clear NOAEL has not been identified, either due to the selection of too high doses or to other deficiencies in the study planning and conduct. In a combined chronic and carcinogenicity study in rats, a LOAEL of systemic, non-neoplastic lesions was 7 mg/kg bw/day. Experience from humans after repeated oral intake also gives indications of haematotoxicity at dosages from 0.4 mg/kg bw/day. The database on repeated-dose toxicity of aniline after inhalation exposure is insufficient, but haematotoxicity at very low concentrations (19-66 mg/m^3) have been reported for rats.

Genotoxicity

The statement regarding the classification and labelling of aniline may need to be revised in the light of new data announced to the CMR Working Group by Industry.

In vitro, aniline has given reproducible negative results in several reverse mutation assays using various *Salmonella typhimurium* strains; however, in the presence of the co-mutagen norharman (present in cigarette smoke and cooked food) and hepatic microsomes, aniline was mutagenic for *Salmonella* strain TA 98. In limited mouse lymphoma assays (no colony sizing was performed to differentiate between gene mutations and chromosomal effects), aniline has shown positive results in the absence of exogenous systems, and conflicting results were obtained in the presence of metabolic activation. Similarly, equivocal results were obtained in a poorly documented HPRT test performed in V79 cells with S-9 mix or with co-cultured rat hepatocytes.

Aniline was clastogenic in CHO, CHL and V79 cells in the presence of a metabolic system, and in CHL and V79 cells at high exposure concentrations also in the absence of

exogenous metabolic activation. A non-adequately reported micronucleus test in SHE cells was negative. Sister chromatic exchanges were produced in the presence and absence of exogenous metabolic activation in CHO cells, and in human fibroblasts and lymphocytes.

Aniline did not induce unscheduled DNA repair in primary human or rat hepatocytes

It had recombinogenic activity in yeast (Brennan, 1997; not mentioned in the RAR) and was positive in the Comet DNA-damage assay (Martin et al, 1999; not mentioned in the RAR)

In vivo, aniline did not induce chromosome aberrations in bone marrow cells of mice dosed with up to 380 mg/kg bw, given twice intraperitoneally (clinical signs of toxicity, but no cytotoxic effect were noted in this study).

In mice, aniline induced micronuclei in several studies, but only at very high dose levels in the toxic range (300-380 mg/kg bw i.p., 1000 mg/kg bw p.o.), whereas in rats a dose-related response in the induction of micronuclei was found in one well performed study. Aniline induced an increased frequency of SCEs in bone marrow cells of mice, dosed intraperitoneally with 220 to 420 mg/kg bw.

Some binding of tritium-labelled aniline to DNA from liver, kidney and spleen was observed after intraperitoneal injection in rats. In another study with either single or repeated intraperitoneal injections, ^{14}C-labelled aniline bound irreversibly to DNA from rat kidney, spleen and large intestine, but no substantial DNA binding was found in rat livers and in mice organs. In alkaline elution assays, DNA strand breaks have been observed in rat liver and rat and mice kidneys, but not in rat spleen or other organs of mice after intraperitoneal administration.

An increase in non-disjunction, but no sex-linked lethals or translocations, were induced in Drosophila (Munoz et al., 1998; not mentioned in the RAR)

Negative results have been found in the analysis of sperm head anomalies and equivocal results in the dominant lethal test. Due to a lack of sensitivity of these tests, the information is insufficient for a sound evaluation of the potential of aniline to induce germ cell mutations.

Carcinogenicity

Aniline is currently classified as carcinogenic, category 3 and labelled with R40 "limited evidence of a carcinogenic effect"

Human carcinogenicity data

Available epidemiological data are inadequate to allow a conclusion as to the carcinogenicity of aniline in humans. Cases of bladder tumours among aniline dye workers are reported. However, these workers were generally exposed to a number of different aromatic amines including aniline, alpha- and beta-naphthylamine, benzidine and auramine, and there is not sufficient evidence to suggest that aniline itself has caused the bladder tumours.

Animal data

Carcinogenicity studies in B6C3F1 mice showed that oral administration of 6,000 or 12,000 mg/kg aniline hydrochloride for 103 weeks produced no significant increases in any type of tumour.

A dose-related increase in the incidence of fibrosarcomas and sarcoma of the spleen and abdominal cavity have been reported for Fischer 344 rats administered 3,000 or 6,000 mg/kg aniline hydrochloride in the diet. Dietary administration of aniline hydrochloride for two years to F 344 rats at levels of 0, 200, 600 and 2000 ppm produced an increased incidence of various types of splenic sarcomas in male rats in the high-dose group. Stromal hyperplasia and fibrosis of the splenic red pulp, which may represent a precursor lesion of sarcoma, were also observed in the high-dose males and, to a lesser degree, in female rats.

No tumours were observed in the bladder, liver, spleen or kidney (the only organs evaluated) after life-time exposure of rats to 22 mg/day aniline hydrochloride in the drinking water and in another limited study in Wistar rats given 0, 0.03, 0.06 or 0.12% aniline alone or in combination with norharman in the drinking water for 80 weeks.

In vitro cell transformation tests with aniline gave inconclusive results.

Reproductive toxicity

Multi-generation or other fertility studies have not been performed with aniline. In the 104-week chronic toxicity aniline feeding study in Fischer 344 rats, no evidence of toxicity of the male reproductive system was observed. In the females of this study, ovary weights were reduced in the high (72 mg/kg bw/day) dose group. In the mouse bioassay with B6C4F1 mice, no treatment related effects in any of the organs of the reproductive system of either males or females were reported.

Aniline has been evaluated for teratogenicity as well as for postnatal effects in an oral study with Fischer 344 rats. At the highest dose of 100 mg aniline hydrochloride/kg bw/day, signs of haematotoxicity were revealed for the dams. A statistically significant increase in relative spleen weight of the dams during the period of gestation was seen at the lowest dose (10 mg aniline hydrochloride corresponding to 7 mg aniline/kg bw/day) was found, so that a NOAEL could not be identified for the dams. Foetuses, as well as newborns, of the high dose treated dams exhibited minor indications for changes in haematological parameters, indicating a NOAEL for developmental toxicity of 30 mg aniline hydrochloride, corresponding to 21 mg aniline/kg bw/day.

3. RISK CHARACTERISATION

Workers

Acute toxicity

The critical exposure levels for eliciting acute toxic effects have been identified as being 1.5 mg/m^3 for 8 hr inhalation and 15 mg per person and day for dermal exposure. The following exposure scenarios give rise to concern: 1) Production and further processing in the large scale chemical industry; 2) iron, steel and aluminium production; and 3) use of dyes with residual aniline.

Irritation/corrosion

Given that control measures exist which should be able to efficiently minimise exposure, the proposed conclusion ii) for irritation seems justified.

Sensitisation

Since there are reports that aniline may cause sensitisation in humans, the possibility of workers to develop contact allergy is of concern (conclusion iii). This is especially the case during production and further processing in the large-scale chemical industry when using unsuitable gloves and use of dyes with residual aniline. Also, the possibility that aniline could cause respiratory sensitisation warrants conclusion (iii). This is in contrast to the conclusion (ii) in the RAR.

Repeated-dose toxicity

The assessment has used the oral LOAEL of 7 mg/kg bw/d and inhalation LOAEC of 17 ppm from rats, corresponding to a human oral dose of 490 mg/person/day and a workplace air concentration of 49 mg/m^3. In addition, an adjustment factor of 3 is being used to adjust from LOAEL to NAEL and a composite uncertainty factor of 50 has been applied. This leads to a critical exposure level of 4.6 mg/person/day or 0.5 mg/m^3. The RAR proposal of conclusion iii) for 1) production and further processing in the large-scale chemical industry; 2) vulcanisation or rubber plastics and rubber processing; 3) iron, steel and aluminium foundries; and 4) use of dyes with residual aniline, is supported.

Genotoxicity

Aniline induced chromosomal effects in several *in vitro* tests and induced micronuclei in rats, and, at high and toxic doses, in mice. DNA adducts have been found in various organs of rats, but not in mice.

The CSTEE agrees with the view that overall the findings from genotoxicity studies are difficult to interpret and that the available information is insufficient for evaluating the mutagenic potential in germ cells. The member states' rapporteur does not propose further testing, as it is not expected to clarify the remaining questions and makes note of the high uncertainty associated with the evaluation of this endpoint.

The CSTEE agrees with the conclusion iii) for all occupational and consumer scenarios since a genotoxic effect cannot be excluded based on the available information.

Carcinogenicity

Aniline induced tumours of the spleen in two studies with rats, and there is some supporting evidence for a genotoxic effect of aniline. No splenic or other tumour types were seen in mice. The mechanism of tumour formation and its relevance for humans is presently unclear. In particular, there is not sufficient evidence to postulate a threshold mechanism and hence aniline was considered to be a non-threshold carcinogen by the member states' rapporteur. It is possible that erythrocyte toxicity and ensuing splenic toxicity may afford a promotive stimulus for tumour formation. However, since a genotoxic mechanism cannot be discounted, the CSTEE supports the conclusion that aniline cannot be classified as a threshold-type carcinogen.

Since tumour incidence was non-linear in the rat studies, a multistage model was applied and a risk level of 1 x 10^{-4} was used as a decision mark between situations for which immediate action is deemed necessary and those for which a low risk level should be taken into account when considering the adequacy of the existing control measures.

From the CIIT rat carcinogenicity study, a T25 dose level of 46 mg/kg/day was obtained, corresponding to 3220 mg/person and day and an air concentration of 322 mg/m^3. With

an interspecies extrapolation factor of 10 and correction for the human "standard life span" and occupational exposure, a minimal MOE of 1620 was calculated for a life-time cancer risk level of 1×10^{-4}.This corresponds to critical exposure levels of 2 mg/person and day (3220/1620), or 0.2 mg/m^3 (322/1620). These calculations assume linearity, an assumption that can be challenged, especially in view of the uncertain genotoxicity of aniline.

Several working areas were identified as being of high concern (production and further processing in the large-scale chemical industry, foundries with partial open systems, vulcanisation of rubber plastics, rubber processing and use of dyes with residual aniline). For the highest exposure scenarios combined inhalation and dermal exposures of 6-8 mg/kg/day have been estimated. These exposures represent one tenth of the dose causing splenic tumours in rats.

The CSTEE agrees with the view that there are concerns for all occupational exposure scenarios, though there are uncertainties concerning the mechanism of tumour formation and its relevance for humans. It also supports a risk level of 1×10^{-4} as a "decision mark" between situations for which immediate action is deemed necessary and those for which a low risk level should be taken into account when considering the adequacy of the existing control measures. However, the CSTEE points to the considerable uncertainties underlying the carcinogenic risk assessment of aniline exposure presented in the RAR.

The CSTEE agrees with the conclusion iii) for all occupational scenarios.

Reproductive toxicity

Although fertility studies are lacking, CSTEE agrees with the RAR conclusion that there is no specific concern with respect to fertility up to dose levels which induce chronic toxicity.

The critical exposure level for potential developmental toxicity has been determined to be 150 mg/person/day or 15 mg/m^3. Dermal contact during production and further processing in the large-scale chemical industry in the case of unsuitable gloves leads to conclusion iii).

Consumers

It is not known whether aniline is generally used as a component in consumer products. In Spain, aniline has been a component of a product used for dying shoes. In situations where aniline could occur in consumer products, conclusion iii) for genotoxicity and carcinogenicity would be indicated.

Man exposed indirectly via the environment

Indirect exposure via the environment has been calculated using modelled data for oral intake via food, drinking water and air. Following the local scenario data at a point source, an intake of a total daily dose of 0.74 mg/kg bw/day has been calculated, whereas intake from plant protecting agents has been estimated to 0.11 mg/kg bw/day. Both of these exposures lead to conclusion iii) for repeated dose toxicity, genotoxicity and carcinogenicity, whereas the local scenario also leads to conclusion iii) for developmental toxicity. Although the CSTEE agrees with these conclusions, it is possible that the exposure estimates are conservative since they are based on modelled data.

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