Acrylonitrile

MAK value	**–**
Peak limitation	**–**
Absorption through the skin (1958)	**H**
Sensitization (1999)	**Sh**
Carcinogenicity (1977)	**Category 2**
Prenatal toxicity	**–**
Germ cell mutagenicity	**–**
BAT value	**–**
Synonyms:	cyanoethylene propenenitrile vinyl cyanide
Chemical name (CAS):	2-propenenitrile
CAS number:	107-13-1
Structural formula:	CH_2=CH–CN
Molecular formula:	C_3H_3N
Molecular weight:	53.06
Melting point:	– 83°C
Boiling point:	77.3°C
Vapour pressure at 20°C:	115.8 hPa
1 ml/m^3 (ppm) ≙ 2.205 mg/m^3	**1 mg/m^3 ≙ 0.453 ml/m^3 (ppm)**

1 Toxic Effects and Mode of Action

Acrylonitrile causes the same signs of toxicity as hydrogen cyanide, but the onset of the effects is clearly delayed due to the metabolism. Acrylonitrile is rapidly absorbed after oral, dermal or inhalative administration and distributed throughout the entire body. As a liquid or vapour it is highly irritating to the skin and slightly irritating to the eye.

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Agitation, convulsions, depression, limited activity, constricted pupils, diarrhoea, hyperuresis, breathing difficulties and even apnoea, erythema, and lacrimation, salivation and nasal discharge develop after acute inhalative, oral and subcutaneous administration. Organ changes are observed in the liver, adrenal gland, brain and pituitary gland. Acrylonitrile causes tumours in the brain, Zymbal gland and forestomach after long-term oral or inhalative administration to rats and thereby proves to be carcinogenic in animal studies. The genotoxic effects are unclear and can not be conclusively evaluated; however, genotoxicity is not to be ruled out, as the mechanism which resulted in the positive findings is not clarified. Cyanoethylene oxide, the metabolite formed, indicates at least a mutagenic potential for acrylonitrile. The metabolite is detectable *in vivo* in blood and in the brain, but a clear genotoxic effect does not occur in *in vivo* studies. Cyanoethylene oxide proved to be mutagenic in two of three *in vitro* experiments.

Acrylonitrile is sensitising to the skin.

2 Mechanism of Action

As a directly alkylating agent, acrylonitrile reacts via addition preferably with strongly nucleophilic centres, for example the SH groups of proteins or the tripeptide glutathione. After administration of [2,3-^{14}C]acrylonitrile (0.1–28 mg/kg body weight) by gavage, the amounts of haemoglobin adducts detected in rats were higher than in mice. *S*-(2-carboxyethyl)cysteine, which is formed by hydrolysis of *S*-(2-cyanoethyl)cystine, which in turn is formed by a direct reaction of acrylonitrile with cysteine in globulin, was measured as an adduct (Fennell *et al.* 1991a). After subcutaneous injection of [2,3-^{14}C]acrylonitrile (1.2–115 mg/kg body weight), time- and dose-dependent acrylonitrile–globulin adducts were measured in rats. *N*-(2-cyanoethyl)valine was quantified as an adduct (Benz *et al.* 1997a).

Cyanoethylene oxide formed oxidatively by cytochrome P450 enzymes is even more reactive than acrylonitrile and also reacts with weaker nucleophiles, e.g. disulfide and hydroxyl groups in proteins or nucleophilic centres in nucleobases (Peter and Bolt 1984). Conjugation of acrylonitrile and cyanoethylene oxide with glutathione led to a decrease of the glutathione level in a range of 30–60% in the liver, kidneys, brain and lungs (Cote *et al.* 1984; Gut *et al.* 1985; Haskovec *et al.* 1988; Vodicka *et al.* 1990). This resulted in disturbances of redox processes in the cell and an increased binding of acrylonitrile and cyanoethylene oxide to macromolecular structures such as cell proteins and nucleic acids. Effects on the redox systems in the cell were found in acute studies, for example in the form of increased lipid peroxidation in hepatocytes (Ivanov *et al.* 1989; Nerudová *et al.* 1988), increased lung toxicity of pure oxygen (Vilim *et al.* 1988) and disturbances of membrane-bound 2,3-diphosphoglycerate mutase and adenosine triphosphatase in the erythrocytes (Farooqui and Ahmed 1983a; Farooqui *et al.* 1990). The adverse effect on the gastric mucosa detected in some acute studies after subcutaneous or oral administration (Ghanayem and Ahmed 1983, 1986; Ghanayem *et al.* 1983, 1985) might

be due to the effect of acrylonitrile or its metabolites on the acetylcholine muscarine receptors after depletion of the glutathione supply (Ghanayem and Ahmed 1986). The neurotoxic changes correlating with increased acetylcholine values are attributed to an inactivation of acetylcholinesterase by the binding of acrylonitrile to this enzyme (Peter and Bolt 1984). The carcinogenicity of acrylonitrile found after chronic administration in animal studies and the genotoxic potential detected *in vitro* might be explained by the binding of the intermediately formed cyanoethylene oxide to nucleophilic centres of the RNA and DNA (Peter and Bolt 1984).

In addition, toxicity mainly in mice is also determined by the cyanide ion released intermediately, which leads to inhibition of cytochrome oxidase. The cyanide ion concentration detected in the brain in the lethal dose range for acrylonitrile approximately corresponded to that after administration of lethal potassium cyanide doses (Tanii and Hashimoto 1984).

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution and elimination

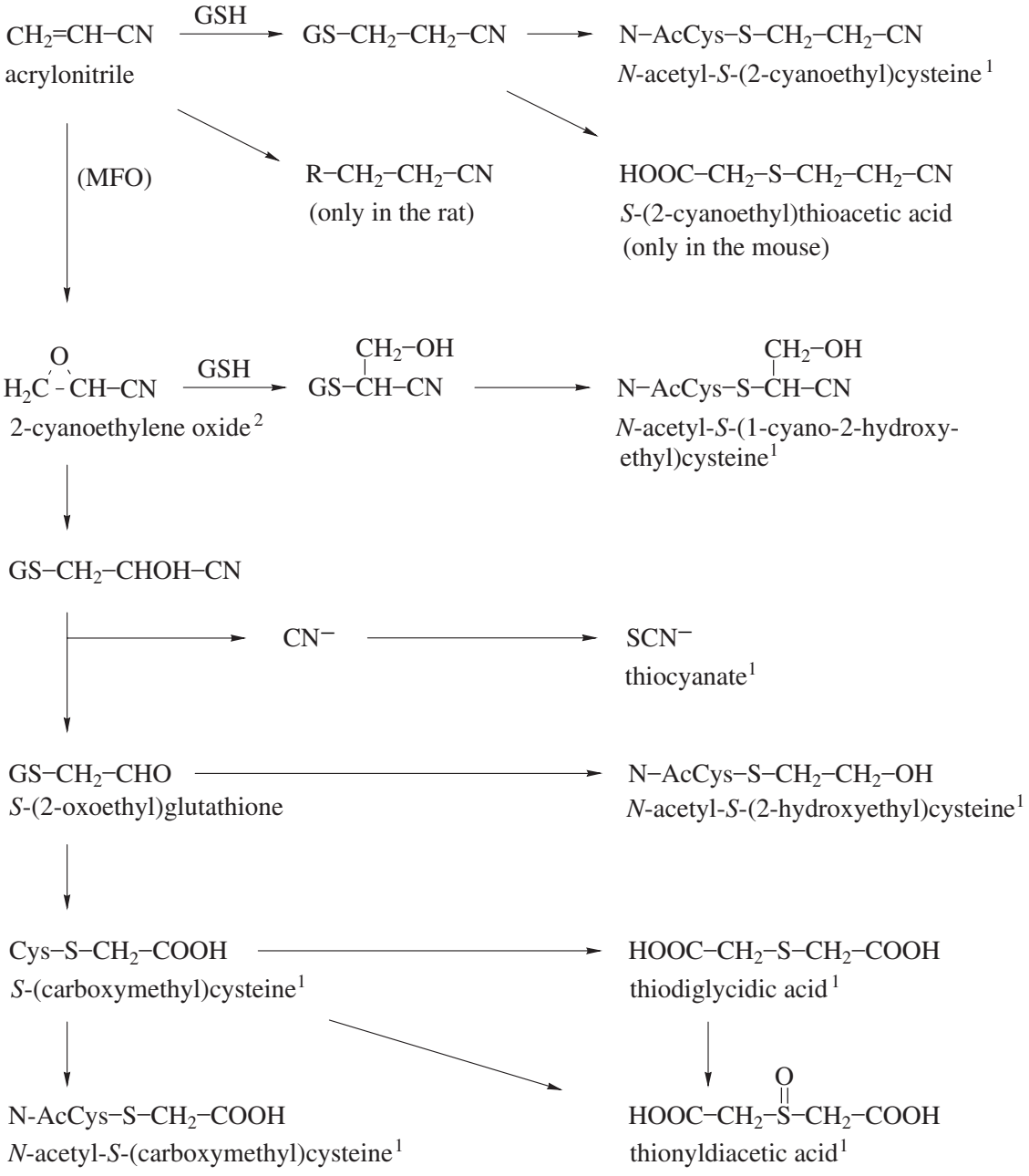
Humans

In an inhalation study with male volunteers, an absorption rate of an average of 52% was detected after 8-hour exposure to acrylonitrile concentrations of 5 and 10 mg/m^3 (Jakubowski *et al.* 1987). *In vitro*, penetration rates of 0.033 and 0.066 mg/cm^2 and minute were determined for the skin after 30-minute and 60-minute applications, respectively (Bakker *et al.* 1991).

Rats and mice

Acrylonitrile is rapidly absorbed and distributed in the whole body after oral administration, dermal application or inhalation (BUA 1995). 3–6 male F344 rats received a single dose of 46 mg 2[^{14}C]acrylonitrile/kg body weight by gavage. After 24 hours, about 10% of the administered dose was measured in the blood, 11% in the faeces and 67% in the urine, and another 11% was exhaled as CO_2. The radioactivity measured in the brain was about 10% of that in the blood (Burka *et al.* 1994). In mice, too, 57–94% was excreted in the urine and less than 8% in the faeces after 24 hours (Kedderis *et al.* 1993c).

3.2 Metabolism



1 detected in the urine of rats and mice
2 detected *in vitro*: liver and lung microsomes of humans; liver, lung and brain microsomes of rats and mice; *in vivo*: blood and brain

Figure 1. Metabolism of acrylonitrile (Fennell *et al.* 1991; Kedderis *et al.* 1993c)

Humans

The metabolites *N*-acetyl-*S*-(2-cyanoethyl)cysteine or *N*-acetyl-*S*-(1-cyano-2-hydroxyethyl)cysteine were determined in liver microsomes and liver cytosol after acrylonitrile or cyanoethylene oxide had been added. During the enzymatic conjugation of acrylonitrile, more product was formed in the cytosolic fraction than during the non-enzymatic conjugation. No difference was found for the conjugation of cyanoethylene oxide (Kedderis *et al.* 1995). In liver and lung microsomes, acrylonitrile is oxidized to cyanoethylene oxide catalyzed mainly by cytochrome P450 2E1 (Guengerich *et al.* 1991; Kedderis *et al.* 1993b) with the oxidation kinetics being similar to those of rats (Roberts *et al.* 1991). Cyanoethylene oxide is hydrolyzed non-enzymatically. A clear increase in the hydrolysis of cyanoethylene oxide was observed *in vitro* after the addition of liver microsomes, but not of cytosol. A K_m value of 0.6–3.2 mM and a V_{max} value of 8.3–18.8 nmol/min and mg protein were specified for the hydrolysis of cyanoethylene oxide by microsomal epoxide hydrolase. The formation of the hydrolysis products was not investigated further. Since no increase in the hydrolysis product was detected after mouse and rat liver microsomes or cytosol had been added, this indicates an additional detoxification pathway for cyanoethylene oxide in humans (Kedderis and Batra 1991a, 1991b, 1993).

Rats

Acrylonitrile is conjugated with glutathione both enzymatically and non-enzymatically. In studies with rat brain cytosol, the non-enzymatic conjugation was higher. In contrast, the enzymatic conjugation of acrylonitrile and cyanoethylene oxide with glutathione was higher in rat liver microsomes and rat liver cytosol than the non-enzymatic conjugation (Kedderis and Batra 1991a, 1991b; Kedderis *et al.* 1995). *N*-acetyl-*S*-(cyanoethyl)-cysteine was detected as the main metabolite of the reductive metabolism in the rat urine. *In vitro*, acrylonitrile is oxidatively metabolized to cyanoethylene oxide at cytochrome P450 2E1, which was detected *in vitro* in liver, lung and brain microsomes. Cyanoethylene oxide is conjugated or hydrolyzed with glutathione. No increase in hydrolysis was observed *in vitro* after the addition of brain microsomes or cytosol. It has not been possible so far to identify the hydrolysis products and detect them *in vivo*. Glutathione deficiency promotes the oxidative metabolism. Cyanoethylene oxide that is formed is distributed uniformly in all tissues of the body and does not only accumulate preferentially in the tissues in which tumours occur. Cyanoethylene oxide is a stable epoxide under physiological conditions with a half-life of 99 minutes at 37°C in 0.1 M sodium phosphate buffer (pH 7.3) (Kedderis and Batra 1993). Cyanoethylene oxide was detected after 5–10 minutes in the blood (428 pmol/ml) and brain (433 pmol/g) after oral administration of 10 mg acrylonitrile/kg body weight. The cyanoethylene oxide concentration in the blood was increased linearly after oral administration of 1, 4, 10 and 30 mg acrylonitrile/kg body weight. There was no saturation at 30 mg/kg body weight (Roberts *et al.* 1991). After oral administration, *N*-acetyl-*S*-(2-hydroxyethyl)cysteine and *N*-acetyl-*S*-(1-cyano-2-hydroxyethyl)cysteine were the main metabolites detected in the urine and *N*-acetyl-*S*-(carboxymethyl)cysteine, thiodiglycolic acid, thionyl diacetic acid and thiocyanate were found as further metabolites (Kedderis *et al.* 1993a). The fraction

of metabolites formed oxidatively after oral administration is 60% in the urine of rats and 80% in the urine of mice (Fennell *et al.* 1991b).

4 Effects in Humans

4.1 Single exposures

Some accidental poisonings with acrylonitrile have been described; most of these were caused by inhalation of high concentrations or prolonged skin contact. Deaths were also observed sporadically (Brieger *et al.* 1952).

Intoxication after occupational contact with acrylonitrile was generally slight (Brieger *et al.* 1952); this was confirmed by more recent data (Zeller *et al.* 1969). Slight icterus, anaemia and unclear general symptoms (Brieger *et al.* 1952) and nausea, vomiting, headache and dizziness (Zeller *et al.* 1969) were described as signs of toxicity. Exposure of the skin to acrylonitrile caused a burning sensation, erythema after some hours and blister formation only after a prolonged period – often only on the following day. These symptoms were also observed when there was no direct contact of acrylonitrile with the skin, but through clothes (Zeller *et al.* 1969).

4.2 Repeated exposures

The activities of acid phosphatase, myeloperoxidase and succinate dehydrogenase were reduced in leukocytes of the peripheral blood of workers, some of whom had been exposed to acrylonitrile for more than 10 years. The glycogen content was increased (no other details) (Grigoreva 1990).

Acrylonitrile–haemoglobin adduct levels were measured in a range of 0.02–66 nmol/g haemoglobin among all 41 workers who had been exposed to acrylonitrile in the production of acrylamide. The publication includes no data for smoking habits (Bergmark *et al.* 1993).

4.3 Local effects on skin and mucous membranes

50 cases of skin lesions were described after dermal exposure to acrylonitrile. A burning sensation on the skin developed after 5 minutes and blister formation occurred after one day (no other details) (WHO 1983).

4.4 Allergenic effects

A 27-year-old man developed a rash on his finger after having worn a splint of a copolymer consisting of acrylonitrile/methyl methacrylate for 6 weeks. Positive results were found in the patch test with the copolymer and 0.1% acrylonitrile (Balda 1975).

5 workers who were involved in the production of acrylonitrile developed lesions on the skin which were attributed to the contact with acrylonitrile. In the patch test with 0.1% acrylonitrile solution (99.5% purity) in petrolatum, all patients reacted with a severity of 3 +. The 8 control persons did not show any reaction on the skin (Bakker *et al.* 1991).

4.5 Carcinogenicity

According to epidemiological studies carried out by the U.S. industry, there seems to be an increased risk for lung and colon carcinomas among workers with potential exposure to acrylonitrile. A cohort of 470 male workers who started to work in the polymerization process of a textile fibre plant between 1950 and 1955 was investigated. The analysis of the data up to 1975, corresponding to a latency period of 20 years, had the following results: 16 cases of cancer (living and dead) were found among the workers exposed. On account of the special situation, however, only 5.8 cases were expected and 6.9 on a national level. 6 cases were lung cancers at a rate of 1.5 expected and 3 cases were colon cancers at a rate of 0.5 expected. The remaining 7 cases had cancers with a different primary localization. All cases were found among workers who had been exposed in the start-up stage of production between 1950 and 1952. No cancers were observed among the workers who were exposed from 1953–55 for the first time (about 25% of the employees examined).

According to the mortality data for the active workers and pensioners of the same group (exposure between 1950 and 1952), 8 deaths from cancer occurred. However, only 4 cases of cancer were expected in the same period according to the mortality figures of the specific company and 5.1 deaths from cancer according to the statistics for the whole of the United States in 1970. 4 of the 8 persons who died from cancer had lung cancer, and the expected rate was 1.5 (Dupont de Nemous Co. 1977).

The results of 25 epidemiological studies were summarized by Collins and Acquavella (1998). The review is an extension of a meta-analysis, which Rothman (1994) had published some years before. The meta-analysis by Collins and Acquavella (1998) did not reveal any increased mortality or elevated relative risks for the formation of tumours. Higher relative risks for the formation of tumours were observed in some organs. The relative risk of dying from bladder cancer was increased as a whole (1.4; 95% confidence interval (CI): 0.9–2.0), but no dose-response relationship was found and the risk was limited to the workers involved in the production of aromatic amines. There are two individual studies with data for the incidence of the formation of Hodgkin's lymphoma. With 2.4 (95% CI: 0.7–8.0), the relative risk is increased, but it is based on only 4 cases. The number of cases observed with a tumour (783 persons that died and 118 persons affected) is so high that the carcinogenicity will probably not be assessed very

differently if new studies are carried out. It is assumed that the acrylonitrile concentrations were in a range of 1.6–22 ml/m^3 (odour threshold) or clearly above in some cases, since workers often perceived the odour of acrylonitrile. In most studies, however, exact data for the exposure to acrylonitrile are not given. No significantly increased relative risks for the formation of tumours were observed in the individual studies either. In most studies, further confounders such as smoking and co-exposure to butadiene were not taken into account so that it is difficult to derive a conclusive evaluation of the carcinogenicity for humans from the epidemiological studies.

5 Animal Experiments and *in vitro* Studies

5.1 Acute toxicity

Excitation, convulsions, depression, reduced activity, contracted pupils, diarrhoea, polyuria, respiratory disorders and even respiratory arrest, erythema, lacrimation, salivation and nasal discharge were found after inhalation, oral administration and subcutaneous application in acute studies. Organ changes were observed in the liver, adrenals, brain and pituitary. The gastric mucosa showed necroses in relation to the dose. Dose-dependent haemorrhages were observed in the gastrointestinal tract. Changes in the renal tubules were found histopathologically. Neurotoxicity was the main effect in rats, whereas no neurotoxic effects were observed in mice. The oral LD_{50} values for various species were in a range of 25–186 mg/kg body weight. The dermal LD_{50} values were determined to be in a range of 148–693 mg/kg body weight for rats, guinea pigs and rabbits. The LC_{50} values were in a concentration range of 300–990 mg/m^3. A decrease of glutathione down to 30–60% was measured in the liver, blood, lungs and brain. The glucose level increased in the blood (BUA 1995; WHO 1983). All of 15 rabbits died after dermal application of 200 mg/kg body weight. No clinical effects were observed (Vernon *et al.* 1990).

5.2 Subacute, subchronic and chronic toxicity

Reduced body weight, necroses of the liver, degenerative changes of the renal tubules, nephritis, hyperplasia of the gastric mucosa, glutathione depletion in the liver, weakly pronounced duodenal ulcers, bronchopneumonia and effects on the CNS occurred after repeated administration of acrylonitrile in rats. Both the statements about the effects on the adrenals and adrenal cortex and about the increase in liver weight are inconsistent (Table 1). An increased coagulation capacity of alveolar macrophages of the lungs after exposure to acrylonitrile was assessed as evidence of a lung-damaging effect. Most of the studies shown in Table 1 have been inadequately documented. Some of the more

recent studies with inhalation were carried out with only one concentration. The studies are therefore not appropriate for the assessment of the chronic toxicity of acrylonitrile.

The following three studies are relevant for assessment and are therefore described in detail:

In an inhalation study by the Chemicals Manufacturing Association (CMA 1980a; for study design see Table 6), the body weight decreased from 44 mg/m^3 in the female rats and from 176 mg/m^3 in the males. A significantly increased mortality was observed at 176 mg/m^3 both in males and in females compared with the control group. Water consumption was increased and the specific gravity of the urine was correspondingly lower during the first 6 months. The haemoglobin content and the number of erythrocytes and leukocytes were reduced. 40 organs of the animals of the control group and of the 176 mg/m^3 group were examined histopathologically. In the males, pathological changes were observed in the lungs in the form of pneumonia starting at 44 mg/m^3 and in the teeth starting at 176 mg/m^3. The histopathological changes observed in the heart and lungs of the animals exposed were identical with those in the control animals, which is attributed to the high incidence of chronic kidney disorders both in the animals exposed and in the control animals. In the females, extramedullary haemato-poiesis in the spleen and liver was significantly increased and liver necroses were observed from the lowest dose. After exposure to both 44 mg/m^3 and 176 mg/m^3, inflammatory and degenerative changes of the nasal turbinates occurred, which were characterized as rhinitis, focal erosions and hyperplasia and metaplasia of the respiratory epithelium and of the mucous-secreting cells. These effects were more pronounced at 176 mg/m^3 than at 44 mg/m^3 and were attributed to the irritation caused by acrylonitrile. Hyperplasia of the mediastinal lymph nodes was observed at 176 mg/m^3. Non-neoplastic changes such as perivascular accumulation of leukocytes and gliosis occurred in relation to the concentration. A no observed effect level (NOEL) for inhalation exposure cannot be derived from this study.

In a study carried out by the U.S. Southern Research Institute in 1996, groups of 10 male and 10 female B6C3F1 mice were given 1.2, 2.4, 4.8, 9.6 and 12 mg/kg body weight by gavage 5 days/week for 13 weeks. Effects on the blood count occurred, but were not related to the dose and were within the range of biological variation so that the authors did not consider them to be relevant for assessment. Mortality, body weight and sperm morphology were unchanged, and no clinical or histopathological effects were observed. A NOEL of 12 mg/kg body weight after oral administration to mice can be derived from this study (Hazardous Substances Assessment Unit Health and Safety Authority 1998).

In a study carried out by the Monsanto Company (1980), groups of 100 male and 100 female F344 rats were exposed to 1, 3, 10, 30 and 100 mg/l (♂: 0.1, 0.3, 0.8, 2.5 and 8.4; ♀: 0.1, 0.4, 1.3, 3.7 and 10.9 mg/kg body weight) of acrylonitrile in the drinking water. Body weight decreased from 2.5 mg/kg body weight in the males and at 10.9 mg/kg body weight in the females. Feed consumption was reduced in the females and water consumption was decreased in the males and females at 8.4 and 10.9 mg/kg body weight, respectively. The relative liver, kidney and heart weights were increased in the males at 8.4 mg/kg body weight, and the liver and heart weights were elevated in the females from 3.7 mg/kg body weight. The authors clearly assign this effect to the test

substance although body weight was reduced. The haemoglobin content, the haematocrit and the number of erythrocytes decreased in the females given 10.9 mg/kg body weight. The alkaline phosphatase activity in the serum was lower in the females from 1.3 mg/kg body weight and in the males from 8.4 mg/kg body weight. An increase in the specific gravity of the urine was observed in the males from 8.4 mg/kg body weight. An oral NOEL of 0.3 mg/kg body weight for rats can be derived from this study (Monsanto Company 1980).

A NOEL of 12 mg/kg body weight for mice and of 0.3 mg/kg body weight for rats can be derived for non-neoplastic effects after oral administration. A NOEL after inhalation exposure cannot be derived since even the lowest concentration tested so far of 44 mg/m^3 led to effects.

Table 1. Studies of the subacute, subchronic and chronic toxicity of acrylonitrile

Species, strain, number of animals, sex	Concentration, dose, duration, route of administration	Effect	References
rat, Wistar, 6 ♂	217 mg/m^3 5 h/d, 5 days, inhalation	increased coagulation capacity of alveolar macrophages of the lungs	Bhooma *et al.* 1992
rat, guinea pig, rabbit, monkey, cat no other details	0–330 mg/m^3 4 h/d, 5 d/week, 8 weeks inhalation	**120 mg/m^3:** no adverse effects **from 220 mg/m^3:** lethargy, body weight reduced, tiredness, weakness of the hind legs, irritation to the nose and eyes, histopathological changes of the liver in cats only; kidney damage, nephritis, bronchopneumonia, congestion and oedema of the alveoli, haemosiderosis in the spleen of rats, haematology: no effects **330 mg/m^3:** mortality increased	WHO 1983
rat, rabbit ♂ and ♀ no other details	0, 50, 240 mg/m^3, 3 h/d, 6 d/week, 6 months inhalation	**from 50 mg/m^3:** eosinophils increased, urine volume increased and protein content increased **240 mg/m^3:** body weight reduced, inflammations of the respiratory tract, degenerations of the proximal tubules; rabbits: heart weight increased	WHO 1983
rat, mouse, dog no other details	0, 58, 117, 234 mg/m^3, 6 h/d, 5 d/week, 13 weeks inhalation	**from 58 mg/m^3:** ataxia, ptosis, rhinitis, increased diuresis, convulsions, body weight reduced, organ weights and haematological parameters: no effects **234 mg/m^3:** mortality increased	WHO 1983
rat, Wistar 15–20 ♂	0, 280 mg/m^3, 8 h/d, 5 days inhalation	relative and absolute liver weights reduced, cytochrome P450 and microsomal proteins in the liver decreased, relative brain weight increased; histopathological examination of lungs, liver, kidneys and adrenals: no effects; glucose in the serum increased, pyruvate and lactate in the blood and brain increased, triglycerides and cholesterol in the serum decreased, GSH depletion in the liver 50%, not in the brain	Gut *et al.* 1984, 1985

Table 1. continued

Species, strain, number of animals, sex	Concentration, dose, duration, route of administration	Effect	References
rat, SD 3–8 ♂	5, 10, 40 mg/kg body weight, 5 d/week, 4 weeks intraperitoneal	**5 mg/kg body weight:** no effects **from 10 mg/kg body weight:** ethane excretion increased, sorbitol dehydrogenase activity increased, body weight reduced **40 mg/kg body weight:** histopathological changes of the liver (necroses, increased mitosis rate and enlarged nuclei), additional treatment with vitamin E prevents increase of sorbitol dehydrogenase activity, ethane excretion increased, body weight reduced	Ivanov *et al.* 1989
rat no other details	0–42 mg/kg body weight, 90 days drinking water	**4 mg/kg body weight:** no organ weight changes, no increased mortality **from 17 mg/kg body weight:** liver weight increased **from 22 mg/kg body weight:** body weight reduced, feed consumption reduced	WHO 1983
dog, beagle 4 ♂ and 4 ♀	0, 100, 200, 300 mg/l (♂: 10, 16, 17; ♀: 8, 17, 18 mg/kg body weight), 6 months drinking water	**from 8/10 mg/kg body weight:** unkempt fur, vomiting, lethargy, weakness, breathing difficulties, kidneys: no effects **from 16/17 mg/kg body weight:** mortality increased, water consumption decreased, feed consumption decreased, brain weight decreased **from 17/18 mg/kg body weight:** haematocrit decreased, erythrocyte count decreased, haemoglobin decreased	WHO 1983

Table 1. continued

Species, strain, number of animals, sex	Concentration, dose, duration, route of administration	Effect	References
rat, SD 48 ♂, 48 ♀	0, 35, 100, 300 mg/l (♂: 0, 3, 9, 21 mg/kg body weight) (♀: 0, 4, 11, 25 mg/kg body weight), 2 years drinking water	uncoordinated movements, tremor, weakness of the extremities, changes in the nervous system ♂ + ♀: **from 3/4mg/kg body weight:** body weight reduced, decrease in feed consumption, decrease in water consumption, hyperplasia of the forestomach, hyperkeratosis **21/25 mg/kg body weight:** anaemia, blood and bile-like fluid in the gastrointestinal tract (effects caused by the tumours) ♀: **from 4 mg/kg body weight:** mortality increased, BUN increased, gliosis and perivascular accumulation of leukocytes in the brain **from 11 mg/kg body weight**: gastric ulcers, hyperaemia **25 mg/kg body weight:** leukocyte count decreased, packed cell volume decreased, Hb decreased, extramedullary haematopoiesis in the spleen ♂: **from 3 mg/kg body weight:** Hb decreased, specific gravity of the urine increased **from 9 mg/kg body weight**: BUN increased, keratitic epidermal inclusion cysts in the skin **21 mg/kg body weight:** mortality increased, endocardial fibrosis, gliosis and perivascular accumulation of leukocytes in the brain, increase in incidence of mediastinal fatty atrophy, dysgnathia	CMA 1980b
rat, SD 3–5 ♀	429 mg/kg body weight 3 times daily for 4 days subcutaneously or orally	weakly pronounced duodenal ulcers, moderately pronounced necrotic changes of the adrenals, effects on the CNS and some organs (no other details), lungs congested and oedematous (inadequate documentation)	Szabo *et al.* 1982

Table 1. continued

Species, strain, number of animals, sex	Concentration, dose, duration, route of administration	Effect	References
rat, SD 3–5 ♀	0, 1, 20, 100, 500 mg/l (0.2, 4, 20, 60 mg/kg body weight) 7, 21 and 60 days drinking water 0.2, 4, 20, 60 mg/kg body weight 7, 21 and 60 days gavage	7, 21 and 60 days: histopathological changes of the *zona fasciculata* and *zona reticularis* drinking water: **0.2 mg/kg body weight:** no effects **from 4 mg/kg body weight:** aldosterone plasma concentration decreased **from 20 mg/kg body weight:** hyperplasia of the gastric mucosa, liver weight not dose-dependently increased, corticosteroid level in the plasma decreased **from 60 mg/kg body weight:** body weight reduced, water consumption decreased, kidney enlarged; after 60 days: adrenals: 20–30% decrease in SH groups not bound to protein; gastric mucosa: increase in SH groups not bound to protein gavage: **from 0.2 mg/kg body weight:** adrenal weight reduced (7 days/3 weeks), 50% decrease in corticosteroid level in the plasma **60 mg/kg body weight**: water consumption increased, aldosterone plasma concentration decreased, gastric mucosa: increase in SH groups not bound to protein additional effects after administration by gavage after 60 days: **from 4 mg/kg body weight:** hyperplasia of the adrenal medulla, adrenal weight increased, aldosterone plasma concentration decreased, adrenal gland: 20–30% decrease in SH groups not bound to protein **20 mg/kg body weight:** enlarged kidneys **60 mg/kg body weight:** water consumption increased, liver weight not dose-dependently decreased	Szabo *et al.* 1984

Table 1. continued

Species, strain, number of animals, sex	Concentration, dose, duration, route of administration	Effect	References
rat, SD 3–5 ♀	0, 100, 500, 2000 mg/l (about 20, 60, 100 mg/kg body weight) 2 weeks drinking water; 100 mg/kg body weight by gavage 3 weeks	degenerative changes of the renal tubules in all treated animals, no necroses or haemorrhages drinking water: **from 60 mg/kg body weight:** adrenal weight decreased, atrophy of adrenal cortex, sodium and potassium values in urine increased **from 100 mg/kg body weight:** mortality increased, body weight reduced, water and feed consumption decreased gavage: **100 mg/kg body weight:** body weight reduced, adrenal weight increased, hyperplasia of adrenal cortex, plasma concentrations of corticosteroids and of aldosterone dose-dependently decreased, 24-hour urine dose-dependently decreased, sodium and potassium values in the urine decreased	Szabo *et al.* 1984
rat, SD 12 ♂	12.5, 25, 50 mg/kg body weight, 5 d/week, 12 weeks, observation period 9 weeks, gavage	**from 25 mg/kg body weight:** body weight reduced **from 50 mg/kg body weight:** weakness of the hind legs, salivation, hyperactivity, stereotypies, muscle action potentials increased, conduction velocity decreased (not reversible), nerve action potential decreased (no histopathological examination was carried out)	Gagnaire *et al.* 1998
rat, SD 12 ♂	25, 50, 100 ml/m^3, 6 h/d, 5 d/week, 24 weeks, observation period 7 weeks, inhalation	**from 25ml/m^3:** motor conduction velocity decreased **from 50 ml/m^3:** damp fur, increased salivation, conduction velocity decreased (reversible) **from 100 ml/m^3:** body weight reduced (no histopathological examination was carried out)	Gagnaire *et al.* 1998

BUN: blood urea nitrogen; Hb: haemoglobin

5.3 Local effects on skin and mucous membranes

A single dose of 200 mg/kg body weight was applied occlusively to the intact skin of 15 male rabbits. All animals died within the first 24 hours. No severe necroses were observed (no other details). 0.5 ml acrylonitrile was applied occlusively to two abraded and two intact skin sites on each side of the body of 6 rabbits for 24 hours. The primary irritation index was 7.6 with a maximum value of 8.0. No significant differences between the abraded and intact skin were detected (no other details). 0.1 ml was instilled into one eye of each of six rabbits. The eyes were not rinsed. A maximum irritation index of 35 was determined after 24 hours with a maximum value of 110, and the irritation decreased slightly after 72 hours (22/110) (Vernon *et al.* 1990). Acrylonitrile was thus severely irritating to the skin and slightly irritating to the eyes (see also WHO 1983).

5.4 Allergenic effects

In a maximization test carried out according to the OECD test guideline, acrylonitrile showed a pronounced sensitizing potential in guinea pigs. Reactions were detected in 80, 85 and 95% of the animals after challenge treatment with 0.2, 0.5 and 1% acrylonitrile, respectively (no other details) (Bakker *et al.* 1991).

5.5 Reproductive toxicity

29–39 Sprague-Dawley rats received acrylonitrile doses of 10, 25 and 65 mg/kg body weight orally (by gavage) or were exposed to 40 and 80 ml acrylonitrile/m^3 by inhalation from days 6 to 15 of gestation. Maternally toxic effects that occurred at 65 mg/kg body weight were characterized by increased mortality, excitation, marked salivation, reduced body weight gain, pathological changes of the stomach and increased liver weight. Feed consumption was reduced during the first few days at 25 and 65 mg/kg body weight. After oral administration of 25 mg/kg body weight, reduced body weight gain, short tail and trunk, missing vertebrae, missing pairs of ribs, kidney and anal orifice and retarded ossification were observed in the foetuses. A right-sided aortic arch and anteriorly-placed ovaries were also found. No anomalies occurred at 10 mg/kg body weight. After inhalation, the body weight of the dams and feed consumption were reduced at both concentrations from days 6–8. Water consumption was increased from days 9–20. Short tail and trunk, hemivertebrae, missing vertebrae, omphalocele and retarded ossification were observed in the foetuses of the high concentration group. The authors concluded that the no observed adverse effect level (NOAEL) for rats for embryotoxicity and foetotoxicity is 10 mg/kg body weight orally and 40 ml/m^3 by inhalation (Murray *et al.* 1978). A single intraperitoneal injection of 80 mg/kg body weight into golden hamsters on day 8 of gestation resulted in encephalocele in 7 foetuses of 6 litters. Dyspnoea,

hyperthermia, salivation, wheezing, problems of coordination and opisthotonus were observed in the dams (Willhite *et al.* 1981a, 1981b). Groups of 20 Sprague-Dawley rats were exposed to 12, 25, 50 and 100 ml/m^3 of acrylonitrile for 6 hours/day on gestation days 6–20 (whole-body exposure). Concentrations from 25 ml/m^3 led to concentration-dependent, significant body weight retardation in the dams and foetuses, and 12 ml/m^3 had no effect. No increase of external, skeletal or visceral rates of variation or malformation or any embryotoxic effects were detected in the concentration range tested. Acrylonitrile had no influence on the average number of implantations, live foetuses or resorptions (Saillenfait *et al.* 1993a).

In vitro, acrylonitrile concentrations of more than 152 µM led to concentration-dependent growth retardations and malformations in 10-day-old rat embryos. The embryotoxic effect was reduced after the addition of glutathione and elevated with a metabolic activation system (Saillenfait *et al.* 1992, 1993b). No effects, malformations or disturbances in the behaviour occurred in the offspring of Wistar rats which received 5 mg/kg body weight orally from days 5–21 of gestation. The authors did not draw any conclusions about the relevance of the significant decrease of the monaminoxidase activity or the deviations of the serotonin level in different brain areas (Mehrotra *et al.* 1988). It was shown in a dominant lethal test that the oral administration (gavage) of 60 mg acrylonitrile/kg body weight to male rats for 5 days had no influence on fertility or pre- and postimplantations (Working 1987a, 1987b).

After oral administration of acrylonitrile doses of 1 and 10 mg/kg body weight for 60 days, a significantly reduced sperm count occurred in male CD-1 mice in the high dose group. The pathological examination of the testes revealed degenerative changes of the tubes, cytolysis and pyknosis of the spermatids and interstitial oedema (Tandon *et al.* 1988).

5.6 Genotoxicity

5.6.1 *In vitro*

The studies carried out on the genotoxicity of acrylonitrile in bacteria and mammalian cells *in vitro* are summarized in Tables 2 and 3.

The results of the studies of the mutagenicity of acrylonitrile in the *Salmonella* mutagenicity test are inconsistent and do not reveal a clear pattern of the effect of the substance in this test system. The positive findings described were generally obtained in the presence of a metabolic activation system. As a whole, the data indicate very weak mutagenicity of acrylonitrile in *Salmonella* depending on the metabolism (Table 2).

Table 2. Genotoxicity of acrylonitrile in bacteria

Test system	Concentration	+ S9 mix[1]	Result	References
S. typhimurium TA1535, 1538, 1978	not specified	+ –	positive negative	Milwy and Wolff 1977
S. typhimurium TA98, 100, 1530, 1535, 1950, 1978	about 200 µg/plate	+ –	positive negative	De Meester *et al.* 1979
S. typhimurium TA97, 98, 100, 102	1000, 3200 µg/plate	±	negative	Baker and Bonin 1985
S. typhimurium TA97, 98, 100, 102	0–5000 µg/plate	±	negative	Matsushima *et al.* 1985
S. typhimurium TA97, 98, 100	50–750 µg/ml	±	negative	Brams *et al.* 1987
S. typhimurium TA98, 100	not specified	±	positive	Khudoley *et al.* 1987
S. typhimurium TA98, 100	not specified	±	negative	Knaap *et al.* 1985
S. typhimurium TA97, 98, 100, 1535	10–10000 µg/plate	±	positive in TA100, TA1535 with S9 (up to 30%), negative in TA98	Zeiger and Haworth 1985
S. typhimurium TA98, 100, 1535, 1537, 1538	50–5000 µg/plate toxic at 5000	±	negative	Rexroat and Probst 1985
S. typhimurium TM677	50, 200, 500 µg/ml	±	questionably positive	Liber 1985
Klebsiella pneumoniae (fluctuation test)	not specified	not specified	positive	Knaap *et al.* 1985
S. typhimurium TA1535/ pSK1002 (umu test, induction of an SOS response)	up to 2820 µg/ml	±	negative	Nakamura *et al.* 1987
E. coli PQ27 (SOS chromotest)	0.0053–13300 µg/ml	±	negative	Brams *et al.* 1987

[1] S9 of rats treated with Aroclor 1254 was generally used for metabolic activation as far as this was documented in the primary literature. In the study by Zeiger and Haworth (1985), S9 of hamsters was used in addition to S9 of rats. In the study by Nakamura *et al.* (1987), the rats used for obtaining S9 were not pretreated with polychlorinated biphenyls (Aroclor 1254), but with phenobarbital and benzoflavone.

All studies carried out in different mammalian cell lines to test the ability of acrylonitrile to induce mutations at the thymidine kinase locus showed mutagenic effects of the substance in the presence of a metabolic activation system, whereas both positive and negative results were obtained without metabolic activation. Since the growth properties of the mutants induced were not characterized in these studies, no conclusions

can be drawn as to the responsible mechanism or whether toxic (clastogenic) effects were possibly involved in the development of mutations (Table 3).

In the hypoxanthine guanine phosphoribosyl transferase test in which – unlike in the thymidine kinase test – positive results can hardly be attributed to cytotoxic effects of the test substance, acrylonitrile was mutagenic both in the presence and in the absence of an exogenous metabolic system in metabolically competent fibroblasts (AHH1 cells) and in L5178Y mouse lymphoma cells, but not in V79 cells.

Tests for the induction of micronuclei, chromosome aberrations and sister chromatid exchanges by acrylonitrile in CHO cells (a cell line from Chinese hamster ovary) were generally positive both in the presence and in the absence of an exogenous metabolic system, the positive effects being often associated with cytotoxic effects. Acrylonitrile induced DNA single strand breaks in primary rat hepatocytes, human bronchial epithelial cells and CHO cells; in bronchial epithelial cells, strand breaks also occurred in the absence of obvious cytotoxic effects. In cultivated testicular cells of humans and rats, however, acrylonitrile concentrations of 30–1000 µM caused no strand breaks.

In most of the studies carried out to test the ability of acrylonitrile to induce DNA repair synthesis (UDS) in primary rat hepatocytes and various other mammalian cells, there were no signs of repair induction. Sporadic positive findings originated from studies with questionable methods, in which the DNA repair was determined after inhibition of the replicative DNA synthesis with hydroxyurea by measuring the radioactivity incorporated into the DNA by means of scintillation counting (Table 3).

The ability of acrylonitrile and its metabolite cyanoethylene oxide to alkylate isolated DNA and RNA *in vitro* was detected in numerous studies (Guengerich *et al.* 1986; Hogy and Guengerich 1986; Koch *et al.* 1987, 1988; Pilon *et al.* 1988b; Solomon *et al.* 1984; Solomon and Segal 1985, 1989; Swenberg *et al.* 1988). In the studies carried out with acrylonitrile, alkylation was mainly due to the epoxide cyanoethylene oxide formed intermediately and only to a very limited extent to acrylonitrile itself (Guengerich *et al.* 1986; Hogy and Guengerich 1986). After incubation of cyanoethylene oxide with nucleotides *in vitro*, 2-cyano-2-hydroxyethyl phosphodiesters were identified as reaction products (Yates *et al.* 1994).

In *Saccharomyces cerevisiae*, studies for the induction of gene mutations, mitotic recombination and aneuploidies mainly yielded negative results in the presence and absence of a metabolic activation system (BUA 1995).

Table 3. Genotoxicity of acrylonitrile in mammalian cells *in vitro*

Test system	Concentration µg/ml	S9 mix	Result	References
Gene mutations				
L5178Y/TK mouse lymphoma cells (thymidine kinase)	5–69	+	positive	Amacher and Turner 1985
	22–43	–	positive	
	100–184	±	positive	Lee and Webber 1985
	2.5–40	–	positive	Myhr *et al.* 1985
	1–60	+	positive	Oberly *et al.* 1985
		–	negative	
P388F/TK mouse lymphoma cells (thymidine kinase)	8, 80, 160	+	positive (only at 160)	Anderson and Cross 1985
		–	negative	
TK6, human lymphoblasts (thymidine kinase)	10–50	+	positive (>40 µg/ml)	Crespi *et al.* 1985
	5–20	–	negative	
	27–74	+	positive	Recio and Skopek 1988
	21–80	–	negative	
AHH1, human lymphoblasts (metabolically competent cells) (HPRT)	5–25	+	positive (at 25 µg/ml)	Crespi *et al.* 1985
V79 cells (HPRT)	110–200		negative	Lee and Webber 1985
L5178Y mouse lymphoma cells (HPRT, Na^+/K^+-ATPase)	12.5–200		positive (HPRT), negative (Na^+/K^+-ATPase)	Garner and Campbell 1985
Balb/c-3T3, embryonic mouse fibroblasts (Na^+/K^+-ATPase)	50–200	+	positive	Matthews *et al.* 1985
Chromosome damage				
human bronchial epithelial cells (micronuclei)	25–500	not specified	negative	Hesterberg *et al.* 1988
CHO cells (micronuclei)	531–53100		positive	Douglas *et al.* 1985
CHL cells (chromosome aberrations)	2.5–25	–	positive	Danford 1985
CHL cells (chromosome aberrations)	3.13–25	–	positive (at 12.5 µg/ml)	Ishidate and Sofuni 1985

Table 3. continued

Test system	Concentration µg/ml	S9 mix	Result	References
Chromosome damage				
CHO cells (chromosome aberrations)	53.1–212		positive	Natarajan *et al.* 1985
CHO cells (chromosome aberrations)	1–100 5–100	+ –	positive positive	Gulati *et al.* 1985
rat hepatocytes (chromosome aberrations)	1.25–10	–	negative	Priston and Dean 1985
Sister chromatid exchange				
human bronchial epithelial cells	150, 300, 600	–	positive	Chang *et al.* 1990
CHO cells	53–212	+ –	positive negative	Natarajan *et al.* 1985
	1.6–160 0.16–50	+ –	positive positive	Gulati *et al.* 1985
human lymphocytes	10 1, 10	+ –	negative negative	Obe *et al.* 1985
rat hepatocytes	1.25–10	–	negative	Priston and Dean 1985; Shell Oil Company 1984
DNA single strand breaks				
primary rat hepatocytes (alkaline elution)	68.5–685	–	positive	Bradley 1985
	5.6 mM	–	positive	Martelli *et al.* 1992
human bronchial epithelial cells (alkaline elution)	200, 500	–	positive	Chang *et al.* 1990
CHO cells	531–53100		positive	Douglas *et al.* 1985
(centrifugation in the alkaline sucrose gradient)	not specified		negative	Lakhanisky and Hendrickx 1985
DNA repair				
primary rat hepatocytes (autoradiography)	0.026–530	–	negative	Probst and Hill 1985
	0.1–10000	–	negative	Williams *et al.* 1985
	0.5–531	–	negative	Butterworth *et al.* 1992

Table 3. continued

Test system	Concentration µg/ml	S9 mix	Result	References
DNA repair				
primary rat hepatocytes (scintillation counting)	0.05–531	–	positive	Glauert *et al.* 1985
HeLa cells	not specified		negative	Martin and Campbell 1985
	0.036, 0.18 mM (1.9, 9.6 µg/ml)	–	positive	Rizzi *et al.* 1984
human bronchial epithelial cells (autoradiography)	5–1000	not specified	negative	Hesterberg *et al.* 1988
mammary epithelial cells (autoradiography)	53, 531 (1.0, 10 mM)	not specified	negative	Butterworth *et al.* 1992
Aneuploidy/polyploidy				
CH1-L cells (aneuploidy)	2.5–25	+	negative	Danford 1985
CHL, CH1-L cells (polyploidy)	3.13–12.5	–	negative	Ishidate and Sofuni 1985
rat hepatocytes (polyploidy)	1.25–10	–	negative	Priston and Dean 1985

One positive study of the mutagenicity in human lymphoblasts (thymidine kinase locus) is available for the genotoxicity of cyanoethylene oxide. Cyanoethylene oxide induced DNA repair in human mammary epithelial cells, but not in primary rat hepatocytes. The different result might be due to the different metabolic capacity as to the conjugation with glutathione (Table 4).

Table 4. Genotoxicity of 2-cyanoethylene oxide

Test system	Concentration	S9 mix	Result	References
Gene mutations				
human lymphoblasts	50, 100, 150 µM	–	positive	Recio and Skopek 1988
UDS				
primary rat hepatocytes (autoradiography)	0.01, 0.1, 1 mM	–	negative	Butterworth *et al.* 1992
human mammary epithelial cells (autoradiography)	0.1, 1 mM	–	positive	Butterworth *et al.* 1992

5.6.2 *In vivo*

The studies carried out on the genotoxicity of acrylonitrile *in vivo* are summarized in Table 5.

Table 5. Genotoxicity of acrylonitrile *in vivo*

Species	Test system	Dose	Result	References
Chromosome damage				
mouse	micronucleus test (bone marrow)	20 mg/kg body weight, once, intraperitoneal	negative	Hachiya 1987
mouse, C57Bl/6	chromosome aberration test (bone marrow)	10–45 mg/kg body weight, once, intraperitoneal (45 mg/kg body weight lethal)	negative	Sharief *et al.* 1986
rat, F344	dominant lethal test	60 mg/kg body weight, 5 days, orally	negative	Working *et al.* 1987a, 1987b
DNA repair synthesis				
rat, Sprague-Dawley	lungs, *in vivo/in vivo* test	46.5 mg/kg body weight, once, orally	positive	Ahmed *et al.* 1992a
rat, Sprague-Dawley	spermatocytes, *in vivo/in vivo* test	46.5 mg/kg body weight, once, orally	weakly positive	Ahmed *et al.* 1992b
rat, F344	hepatocytes, *in vivo/in vivo* test	50 mg/kg body weight, once, orally	positive	Hogy and Guengerich 1986; Hogy 1986
	brain, *in vivo/in vivo* test	50 mg/kg body weight, once, orally	negative	
rat, F344	hepatocytes, *in vivo/ex vivo* test, autoradiography	75 mg/kg body weight, once, orally	negative	Butterworth *et al.* 1992
		60 mg/kg body weight, 5 days, orally	negative	
rat, F344	spermatocytes, *in vivo/ex vivo* test, autoradiography	75 mg/kg body weight, once, orally	negative	Butterworth *et al.* 1992
		60 mg/kg body weight, 5 days, orally	negative	
		60 mg/kg body weight, 5 days, orally	negative	Hurtt *et al.* 1987
Sister chromatid exchange (SCE)				
mouse, C57Bl/6	SCE (bone marrow)	10–60 mg/kg body weight, once, intraperitoneal (doses >30 mg/kg body weight lethal)	negative	Sharief *et al.* 1986

Table 5. continued

Species	Test system	Dose	Result	References
DNA single strand breaks				
rat	liver and brain, alkaline elution	not specified	liver positive, brain negative	Hachiya *et al.* 1984
rat	liver DNA, alkaline elution	75 mg/kg body weight, once, intraperitoneal	positive	Hachiya *et al.* 1986
		75 mg/kg body weight, once, intraperitoneal	negative	Hachiya 1987
Drosophila melanogaster				
sex-linked recessive lethal mutation		administration by injection	negative	Knaap *et al.* 1985
sex-linked recessive lethal mutation		not specified	positive	NTP 1987
reciprocal translocations		not specified	negative	NTP 1987
somatic mutations	effects on the eyes using the ZESTE system	53, 106, 212 and 424 µg/ml, in the feed of the larvae	positive at 424 µg/ml	Fujikawa *et al.* 1985
aneuploidy as a result of segregation errors during meiosis		exposure of the females to 131 ml/m^3 for 70 min, mating with untreated males	positive	Osgood *et al.* 1991
somatic mutation and recombination	effects on the eyes using the white-coral somatic colour system	5–20 mM in the feed of the larvae	positive	Vogel 1985
	punctiform changes of the wing pattern in 72-h-old larvae	15.2 mM in the feed, feeding for 2 h or 6 h of the 48- or 72-h-old larvae	negative	Würgler *et al.* 1985
		1.5 mM and 7.6 mM in the feed, feeding up to the pupation of 1-, 48- or 72-h-old larvae	positive	Würgler *et al.* 1985
		exposure of the 48- or 72-h-old larvae to acrylonitrile (0.5 or 1 mg/1150 ml air; 0.5 or 1 h)	weakly positive	Würgler *et al.* 1985

No increases in the incidence of micronuclei, chromosome aberrations or sister chromatid exchanges in the bone marrow were detected in mice after a single intraperitoneal injection. In UDS assays carried out properly as to the method, the single

or repeated administration of acrylonitrile in rats did not lead to an induction of DNA repair synthesis in hepatocytes or spermatocytes. Acrylonitrile induced somatic mutations and recombinations in larvae of *Drosophila melanogaster* after feeding and exposure by inhalation. The authors assessed acrylonitrile as weakly mutagenic since the positive feeding study was only inadequately reproducible in the inhalation test (Würgler *et al.* 1985). No sex-linked recessive lethal mutations were observed in *Drosophila melanogaster* after injection (no other details).

Several studies were carried out to investigate the covalent binding of acrylonitrile or acrylonitrile metabolites in nucleic acids *in vivo*. After oral administration of 46.5 mg [2,3-^{14}C]acrylonitrile/kg body weight in rats, increased radioactivity was found in the isolated nucleic acids of liver, stomach, gastrointestinal tract, brain, lungs and testes. However, no difference was made between the incorporation of radioactive degradation products of acrylonitrile and covalent adducts that may have been formed (Abdel-Rahman *et al.* 1991; Ahmed *et al.* 1991, 1992a, 1992b; Ahmed and Farooqui 1984; Farooqui and Ahmed 1982, 1983b). After oral administration and inhalation of 4 mg [2,3-^{14}C]acrylonitrile/kg body weight to rats, increased radioactivity was measured in the RNA fraction of the stomach, liver and brain. No increased radioactivity was found in the DNA fraction after inhalation, and the data for radioactivity of the gastric DNA after oral administration are inconsistent (Pilon *et al.* 1988a, 1988b, 1988c).

Groups of 3 rats were given acrylonitrile doses of 50 mg/kg body weight and 2-cyano[2,3-^{14}C]ethylene oxide doses of 6 mg/kg body weight by intraperitoneal injection. The animals were sacrificed 2 hours later. The radioactivity of the liver and brain DNA was not increased, nor were any adducts such as N^6-ethenoadenine or N^6-ethenoguanine found (Guengerich *et al.* 1986; Hogy and Guengerich 1986). After long-term administration of acrylonitrile in the drinking water at 500 mg/l (about 50 mg/kg body weight and day), 7-(2-oxoethyl)guanine and N^2,3-ethenoguanine were detected in the DNA of the brain and the Zymbal gland. Low levels of these DNA adducts, which were also formed when incubating calf thymus DNA with acrylonitrile, were found in the liver, too. Only 7-(2-oxoethyl)guanine was found in the gastric DNA. Neither of the two adducts was detected in the spleen DNA (Koch *et al.* 1987, 1988; Swenberg *et al.* 1988). Since the studies mentioned are only available as abstracts, they can be used for assessment only with reservations.

In a further study, groups of 5 Sprague-Dawley rats received acrylonitrile doses of 3, 30 and 300 mg/l (about 0.3, 3 and 30 mg/kg body weight and day) in drinking water for 21 days. A significant increase of the 8-oxodeoxyguanosine level was measured in the brain, liver and forestomach of the animals exposed to 3 and 30 mg/l. The activity of cytochrome oxidase, catalase and glutathione peroxidase in the brain of the animals was not increased. No data were given for cell proliferation in the organs examined or for DNA adducts that were possibly formed (Whysner *et al.* 1998). In a drinking water study also carried out in Sprague-Dawley rats (5, 50, 100 and 200 mg/l; corresponding to 0.6, 5.1, 8.9 and 15 mg/kg body weight; examination times 14, 28 and 90 days), a significant increase in the 8-oxodeoxyguanosine level was measured in the brain, but not in the liver, from 50 mg/l and 90 days of administration. An indication of increased lipid peroxidation measured as malondialdehyde was observed in the brain, but not in the liver, in the highest concentration group only after 14 days. From 50 mg/l, the

glutathione and vitamin E levels significantly decreased only in the brain after 14 days, but not after 90 days. After 14 days, the catalase and superoxide dismutase activities were reduced in the brain from 5 mg/l and 50 mg/l, respectively; after 90 days, they were decreased at 50 mg/l and above and only at 200 mg/l, respectively. A significantly increased release of hydroxyl radicals was detected only in the brain at 50 mg/l and above. The results available to date indicate oxidative stress in the brain as the target organ, but not in the liver (Jiang *et al.* 1998).

5.7 Carcinogenicity

Studies of the carcinogenicity of acrylonitrile are shown in Table 6.

After exposure to acrylonitrile by inhalation at 20 ml/m^3, astrocytomas and tumours of the Zymbal gland were found in rats. Purulent rhinitis, hyperplasia and proliferation of the respiratory epithelium were observed at the same time. The toxic effects can be explained by the severe irritation caused by acrylonitrile. The number of mammary tumours per animal was significantly increased in the low concentration group, but not in the highest concentration group. The absence of a concentration-response relationship might be due to the high mortality rate (CMA 1980a). In a further study, increased tumour incidences were observed in the Zymbal gland from 10 ml/m^3 not related to the concentration and in the brain from 20 ml/m^3 (Maltoni *et al.* 1988) (Table 6). Higher tumour incidences in the brain and the Zymbal gland also occurred in rats after oral administration starting at an acrylonitrile concentration of 10 mg/l (about 1 mg/kg body weight) in the drinking water (Monsanto Company 1980; WHO 1983).

In two studies (Bigner *et al.* 1986; Gallagher *et al.* 1988), these tumours, and forestomach papillomas as well, were only observed at an acrylonitrile dose in the drinking water of 50 mg/kg body weight, at which the survival rate was specified to be 0% (Gallagher *et al.* 1988). In all studies, the survival rate was very low both in the control group and in the groups of exposed animals. Toxic effects were observed in the studies starting at a concentration of 10 mg/kg body weight (Bigner *et al.* 1986; Gallagher *et al.* 1988). On account of small numbers of animals (Gallagher *et al.* 1988) and a lack of histopathological examinations and documentation (Bigner *et al.* 1986), these two studies can be used for assessment only with reservations.

Table 6. Results relating to the carcinogenicity of acrylonitrile

Author:	CMA 1980b
Substance:	acrylonitrile
Species:	rat (Sprague-Dawley), 48/sex and 80 controls/sex
Administration:	in drinking water
Dose:	35, 85 and 210 mg/l for the first 21 days; 35, 100 and 300 mg/l (♂: 3.4, 8.5 and 21.2 mg/kg body weight; ♀: 4.4, 10.8 and 25.0 mg/kg body weight)
Duration:	2 years
Toxicity:	dose-dependent decrease in water and feed consumption; reduced body weight gain; no effects: bone marrow, kidneys, liver

		control	35 ppm	100 ppm	300 ppm
survivors (2 years)	♂	7/80	5/47	5/48	0/48
	♀	20/80	4/48	1/48	0/48
Findings:					
astrocytomas	♂	1/80	8/47[1]	19/48[1]	23/48[1]
	♀	1/80	17/48[1]	22/48[1]	24/48[1]
Zymbal gland tumours	♂	2/80	4/47	3/48	15/48[1]
	♀	1/80	4/48	6/48	13/48[1]
forestomach papillomas	♂	0/80	1/47	10/48 [1]	35/48 [1]
	♀	1/80	1/48	12/48 [1]	29/48 [1]
carcinomas of the tongue	♂	0/80	2/47	3/48	5/48 [1]
small intestine adenocarcinomas	♂	3/80	6/47	1/48	6/48
	♀	0/80	1/48	4/48	4/48 [1]

[1] $p<0.05$

Author:	Monsanto Company 1980
Substance:	acrylonitrile
Species:	rat (F344), 100/sex and 200 controls/sex
Administration:	in drinking water
Dose:	1, 3, 10, 30 and 100 mg/l (♂: 0.08, 0.25, 0.84, 2.49 and 8.36 mg/kg body weight; ♀: 0.12, 0.36, 1.25, 3.65 and 10.89 mg/kg body weight)
Duration:	2 years
Toxicity:	from 100 mg/l in ♀ and ♂: reduced body weight, but no reduced feed consumption; increase in alkaline phosphatase activity; ♀: decrease of the haemoglobin content and erythrocyte count

Table 6. continued

		control	1 mg/l	3 mg/l	10 mg/l	30 mg/l	100 mg/l
survivors (2 years)	♂	92/140	52/70	46/70	37/70	44/70	15/71
	♀	111/140	50/70	46/70	50/70	41/70	15/69
Findings:							
brain/spinal cord:	♂	2/200	2/100	1/100	2/100	10/99 [3]	21/99 [3]
astrocytomas	♀	0/199	1/100	2/100	4/100 [1]	6/99 [2]	24/99 [3]
Zymbal gland:							
adenomas	♂	1/189	1/97	0/93	0/88	2/94	8/93 [3]
	♀	0/193	0/94	1/92	2/90	3/91	1/86
carcinomas	♂	1/189	0/97	0/93	2/88	5/94 [2]	8/93 [3]
	♀	0/93	0/94	1/92	2/90	2/94	7/86 [3]
forestomach:	♂	0/199	1/99	4/93 [2]	4/96 [2]	4/96 [2]	0/100
papillomas	♀	1/198	1/99	2/98	2/95	4/96 [1]	2/95
carcinomas	♂	1/189	0/97	0/93	2/88	5/94 [2]	8/93 [3]
	♀	0/193	0/94	1/92	2/90	2/94	7/86 [3]

[1] $p<0.05$; [2] $p<0.01$; [3] $p<0.001$

Author:	Bigner *et al.* 1986
Substance:	acrylonitrile
Species:	rat (F344), control: 49 ♀ and 51 ♂; group 1: 153 ♀ and 147 ♂; group 2: 50 ♀ and 50 ♂
Administration:	in drinking water
Dose:	group 1: 500 mg/l (about 50 mg/kg body weight) group 2: 100 and 500 mg/l (about 10 and 50 mg/kg body weight)
Duration:	lifetime exposure
Toxicity:	loss of body weight 500 mg/l: after 2–3 weeks; 100 mg/l: after 2 months ♂; in the 2nd year ♀ from 100 mg/l: increased mortality, paralysis, head tilt, circling, seizures, reduced activity and neurological signs: 500 mg/l: 29/300 and 16/100; 100 mg/l: 4/100; control: 0/100, most animals died from brain tumours between months 12 and 18; no other details of survival rate
Findings:	49 of 215 brains examined of the 500 mg/l group had a primary brain tumour; tumours of the Zymbal gland, forestomach papillomas and subcutaneous papillomas at different sites occurred in animals which died between months 6 and 18 (no other details)

Table 6. continued

Author:	Gallagher *et al.* 1988			
Substance:	acrylonitrile			
Species:	rat (Sprague-Dawley), 20/sex			
Administration:	in drinking water			
Dose:	20, 100 and 500 mg/l			
Duration:	2 years			
Toxicity:	increased mortality, body weight loss at 100 and 500 mg/l from month 17; reduced body weight gain at 500 mg/l			
	control	20 mg/l	100 mg/l	500 mg/l
survivors (2 years)	20%	35%	20%	0%
Findings: tumours				
soft tissues	1/18	0/20	0/20	0/20
forestomach	0/18	0/20	0/20	4/20
Zymbal gland	0/18	0/20	1/20	9/20
pituitary	5/18	3/20	1/20	0/20
pancreas	1/18	0/20	2/20	0/20
kidney	0/18	0/20	0/20	1/20
parathyroid	1/18	1/20	0/20	1/20
skin	0/18	0/20	2/20	1/20

Author:	CMA 1980a			
Substance:	acrylonitrile			
Species:	rat (Sprague-Dawley), 100/sex			
Administration:	inhalation			
Concentration:	20 and 80 ml/m^3 (44 mg/m^3 and 176 mg/m^3)			
Duration:	6 h/d, 5 d/week, 2 years			
Toxicity:	from 20 ml/m^3: purulent rhinitis, hyperplasia, focal erosions, metaplasia of the respiratory epithelium and microscopic, metaplastic proliferation of the respiratory epithelium			
		control	20 ml/m^3	80 ml/m^3
survivors				
after 1 year	♂	96/100	92/100	81/100
	♀	91/100	97/100	81/100
after 2 years	♂	8/100	14/100	4/100
	♀	22/100	9/100	1/100

Table 6. continued

Findings				
(multi)focal proliferation of the glia cells	♂	0/100	0/99	7/99 [1]
	♀	0/100	4/100	4/100
astrocytomas	♂	0/100	4/99	15/99 [1]
	♀	0/100	4/100	17/100 [1]
Zymbal gland carcinomas	♂	2/100	4/100	11/100 [1]
	♀	0/100	2/100	13/100 [1]
small intestine carcinomas	♂	2/99	2/20	13/98 [1]
	♀	0/100	3/100	4/100
forestomach papillomas	♂	1/98	2/100	6/99
	♀	not specified	not specified	not specified

[1] $p<0.05$

Author:	Maltoni *et al*. 1988
Substance:	acrylonitrile
Species:	rat (Sprague-Dawley), inhalation: 30/sex; dams: 54; control animals: 54; gavage: 40/sex; control animals (olive oil): 75/sex
Administration:	inhalation and oral (gavage)
Concentration:	inhalation: 5, 10, 20 and 40 ml/m^3, ♂ and ♀ inhalation: 60 ml/m^3 dams and embryos gavage: 5 mg/kg body weight
Duration:	inhalation: 4 h/d, 5 d/week, 52 weeks; inhalation: 4 h/d, 5 d/week, 7 weeks and then 7 h/d, 5 d/week, 97 weeks dams and embryos (group 1); 4 h/d, 5 d/week, 7 weeks and then 7 h/d, 5 d/week, 8 weeks embryos (group 2) gavage: once daily, 3 days/week, 52 weeks
Toxicity:	no increased mortality, no changes in body weight gain and no hepatomas

Findings: inhalation		control	5 ml/m^3	10 ml/m^3	20 ml/m^3	40 ml/m^3
		animals with tumours (%)				
leukaemia	♂	0	0	10	7	10
	♀	0	3	7	3	0
phaeochromocytomas	♂	20	20	27	7	20
	♀	3	17	23 [1]	7	0
phaeochromoblastomas	♂	0	0	10	0	3
	♀	3	0	7	0	3
Zymbal gland	♂	0	0	7	0	0
	♀	0	0	3	3	0
angiosarcomas	♂	3	0	3	0	0
	♀	0	0	0	0	0
brain tumours	♂	0	0	0	3	7
	♀	0	0	0	3	3

[1] $p<0.05$

Table 6. continued

Findings: gavage		control	5 mg/kg body weight
leukaemia	♂	0	5
	♀	9	8
phaeochromocytomas	♂	3	10
	♀	3	3
Zymbal gland carcinomas	♂	0	3
	♀	1	0
brain tumours	♂	1	0
	♀	3	3

Findings: inhalation 60 ml/m^3		control	dams	control	embryos group 1	embryos group 2
mammary tumours benign and malignant/ malignant	♂	–	–	7/2	6/0	2/0
	♀	40/3	69/6	56/5	67/17 [2]	67/7
leukaemia	♂	–	–	8	12	10
	♀	3	9	1	7	10
phaeochromocytomas	♂	–	–	20	18	20
	♀	18	2	18	2	7
Zymbal gland	♂	–	–	1	15 [1]	7
	♀	2	6	0	2	2
extrahepatic angiosarcomas	♂	–	–	1	4	5
	♀	0	2	0	6 [1]	2
hepatomas	♂	–	–	1	8 [1]	2
	♀	0	0	0	2	0
brain tumours	♂	–	–	1	16 [1]	5
	♀	0	6	1	19 [1]	3

[1] $p<0.01$ [2] $p<0.05$

5.8 Other effects

Alveolar macrophages of male Wistar rats were exposed to 0.2, 2, 10 and 20 µM acrylonitrile for 4 hours. The survival rate of the macrophages decreased in relation to the concentration down to 18% at the highest concentration. At 10 µM, the hydrogen peroxide level increased to 44% compared with the control value. After the addition of superoxide dismutase, catalase or EDTA, the survival rate increased again to 80–92% and so protected the cells from the effect of acrylonitrile (Bhooma and Venkataprasad 1997).

6 Manifesto (MAK value/classification)

The epidemiological studies provide no evidence of carcinogenic effects of acrylonitrile, but their validity is limited since important parameters were not taken into account in most studies.

The genotoxicity of acrylonitrile *in vitro* does not reveal a clear pattern and cannot be assessed conclusively. However, it can not be excluded, particularly as the mechanism which led to the positive findings has not been clarified. The genotoxicity assumed to be due to the metabolite cyanoethylene oxide was not detected in *in vivo* studies.

Acrylonitrile caused tumours in the brain, Zymbal gland and forestomach of rats both after oral administration and after inhalation. The studies listed give reason for criticism since the survival rate in the control groups is very low, but the results are mutually supportive, are consistent and thus confirm a carcinogenic effect in animal studies.

The suspicion that toxic effects are involved in tumour formation results from various observations made in the animal studies and also from the intrinsic reactivity of acrylonitrile and its metabolic activation. It is therefore assumed that the changes in certain biochemical end points are not a linear function of the dose. Genotoxic modes of action should also be taken into account in the development of the tumours observed. The metabolite that is formed, cyanoethylene oxide, provides evidence of a mutagenic potential. The metabolism also causes a depletion of glutathione, which in turn causes oxidative stress in the cell.

No NOEL can be derived for inhalation from the previous studies. Data after long-term inhalation exposure in doses between 1 and 20 ml/m^3, information about the mechanism of action and data for the quantitative formation of the genotoxic metabolite cyanoethylene oxide would be necessary to derive a MAK value. It would also be important to know whether external exposure at the workplace results in internal exposure which is significantly higher than that of a reference population. Since there are no data for deriving a NOEL, acrylonitrile cannot be classified in Carcinogen category 4 or 5. Acrylonitrile remains in Carcinogen category 2 of Section III of the *List of MAK and BAT Values*.

Because of the evidence of sensitization, acrylonitrile is designated with “Sh”.

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