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TOXICOLOGICAL EVALUATIONS

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TOXICOLOGICAL EVALUATION

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Trioxane

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CAS No. 110-88-3

BG Chemie
Berufsgenossenschaft der chemischen Industrie

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Trioxane

1 Summary and assessment

Trioxane is absorbed by the body upon oral administration or inhalation exposure. There are no indications from the available data that the chemical is absorbed via the skin. Following oral administration to rats, ^{14}C-trioxane is excreted with a half-life of less than 24 hours. By 72 hours after dosing, 85 to 90% of the chemical has been excreted, with fractions of 72 to 74% being eliminated via the lung as CO_2 and 13 to 15% and 0.7 to 0.8% being excreted in the urine and the faeces, respectively. At that time, 1.8% of the administered radioactivity is recovered in the tissues. Intraperitoneal administration of ^{14}C-trioxane to rats yields comparable results. Elimination from plasma takes place in a biphasic manner consistent with an open two-compartment model with half-lives ranging from 4.5 to 6.8 and 55 to 69 hours. It occurs essentially in the form of CO_2 via the lung. As little as 1 to 2% of the administered radioactivity is excreted in the urine, and only traces in the faeces. Within 72 hours, 8% of the administered trioxane is excreted in the expired air as unchanged substance. At that point, 1.2 to 1.5% of the administered radioactivity is detected in tissues, particularly in the liver and kidneys. When pregnant rats are treated with a single oral dose of ^{14}C-trioxane on day 19 of gestation, excretion also takes place rapidly with a plasma half-life of 14.5 hours. At 24 hours after dosing, only 4.2% of the administered radioactivity is recovered, essentially in the liver and blood plasma of the mothers and in the foetuses. Radioactivity accumulates in the latter over the next 24 hours whereas the levels continue to decline in the maternal tissues. It is assumed that trioxane is essentially hydrolysed to formaldehyde in the body and subsequently metabolised, via formic acid, to CO_2. One study in whole blood from rats demonstrated that 1-hour incubation with trioxane yielded little, if any, conversion of the compound in blood. Fractions of 73 to 93% of the test substance were recovered unchanged.

Trioxane is of very low toxicity following single-dose administration. Oral LD_{50} values reported for rats range from 7740 to 9500 mg/kg body weight. Single dermal treatment of rabbits with 3980 or 15000 mg/kg body weight does not cause death or marked clinical signs of toxicity. Inhalation by rats of trioxane at concentration levels of 26000 or 39100 mg/m³ does not

cause death or marked clinical signs of toxicity. However, mortality in rats is 4 out of 6 following 8-hour inhalation exposure to atmosphere saturated with trioxane at 70 °C. LD_{50} values found in rats and mice after intraperitoneal administration of trioxane are 850 and 1800 mg/kg body weight, on the whole suggesting low acute toxicity despite the great difference between the values.

Single exposure of the intact rabbit skin to trioxane is not observed to cause irritation. However, the chemical causes irritation upon prolonged exposure of the skin or multiple applications to the skin. Trioxane causes slight to severe irritation to the rabbit eye. The effect is reversible.

No skin-sensitising effects occurred following trioxane exposure in several guinea pig studies.

Oral administration by gavage of a daily trioxane dose of 1000 mg/kg body weight to rats for 4 weeks has no effect on body weight gain. The leukocyte count is significantly decreased at the end of treatment, γ-glutamyl transpeptidase activity in the blood is slightly increased while the levels of protein and glucose in the blood are reduced. A lower dose of 200 mg/kg body weight is considered as the *no effect level*. Following oral administration to rats of trioxane at 850 mg/kg body weight for 4 months or at 213 mg/kg body weight for 7 months (both on 5 days/week) no toxic effects are observed other than reduction in body weight gain and, at the high dose, irritation of the gastric mucosa. When rats undergo inhalation exposure to a concentration of 18179 mg/m³ (6 hours/day on 5 days/week for 2 weeks) body weight gain is reduced, and at the end of treatment spleen weight is decreased. The nasal mucosa exhibits squamous metaplasia, the epithelium showing necrosis and desquamation. When rats undergo chronic inhalation exposure to trioxane at 50, 500 or 2500 mg/m³ (5 hours/day, 5 days/week for 12 months), disturbances of motor co-ordination occur which gradually increase with time but are not concentration-dependent. The acetylcholine esterase activity in the blood serum of all treated rats is reduced by approx. 30% at the end of exposure. Epithelial lesions in the trachea occurred at the two higher concentrations. A trioxane concentration of 50 mg/m³ of air is given as the threshold concentration in rats. The toxicological relevance of all results obtained following multiple or chronic administration of trioxane – apart from the effects on body weight development and the mucous membranes of the respiratory tract after inhalation –

must be considered questionable because correlating histopathological findings were lacking in all cases.

Trioxane proved not to be mutagenic in procaryotes when tested for gene mutation in 5 Salmonella/microsome assays. The same was found for V79 cells of the Chinese hamster in an HPRT test and a chromosome aberration test. Only a test in mouse lymphoma cells, when carried out in the presence of a metabolic activation system, yielded a positive result in parallel with very high cytotoxicity. In vivo, a micronucleus test in BALB/c mice and a dominant-lethal test in rats also yielded no indications of a mutagenic effect. Inhalation exposure of male *Drosophila melanogaster* flies to trioxane for 12 days leads to a slight increase in sex-linked recessive lethal mutations, whereas dietary administration of trioxane for 2 to 8 days causes no increase in recessive lethal mutations in male larvae or adult males. Two studies on the effect of trioxane on DNA from rat hepatocytes carried out in vivo have given contradictory results. DNA single-strand breaks are observed in hepatocytes upon single intraperitoneal administration of trioxane at 425 or 850 mg/kg body weight. In contrast, a UDS test involving single oral administration of trioxane doses of up to 2000 mg/kg body weight gives clearly negative results. Overall, trioxane exhibits no genotoxic potential in the valid in-vitro and in-vivo studies.

A cell transformation study in mouse embryo cells did not demonstrate any effect of trioxane on the number of transformed cells, even at higher test concentrations associated with very marked cytotoxicity.

High doses of trioxane result in foetal lethality, retarded foetal growth and congenital malformations in the foetuses. No maternal toxicity was observed upon oral treatment of pregnant rats with trioxane at 770 mg/kg body weight, when administered on alternate days from day 8 to day 20 of gestation. Upon sacrifice on day 21, the animals in that dose group, compared with untreated controls, showed an increase in the number of resorptions, a decrease in foetal body weight and length and malformations of the foetal brain, kidneys and skeletal system. The number of foetuses with delayed ossifications was significantly increased. The 1550 mg/kg dose further increased the observed effects, but also resulted in maternal toxicity. Daily oral administration of trioxane at 190 mg/kg body weight was devoid of any effect. However, this study is of limited value because a number of females from all treatment and control groups were found to

have histopathological changes of the placenta, the influence of which on the emergence of the observed foetotoxic effects is unclear. In a further teratogenicity study conducted in accordance with OECD guideline No. 414, rats received daily oral treatment from day 7 to day 20 of gestation. The dams in the highest dose group (1000 mg/kg body weight) showed a slight reduction in body weight gain. The frequency of resorptions was not affected but dead foetuses were observed. Cases of aplasia of the tail and the vertebral column and delayed ossification of various bones were reported. Even a low dose group (315 mg/kg body weight) exhibited wavy or thickened ribs and retarded ossification. The *no effect level* for embryotoxicity reported in this study was 100 mg/kg body weight. Corrected maternal body weight gain (body weight on day 21 of gestation minus body weight on day 7 of gestation minus gravid uterus weight) was reduced at all dose levels as compared with controls. There was no other maternal toxicity from trioxane. When pregnant rats were treated with an oral trioxane dose of 1160 mg/kg body weight every other day from day 2 to day 20 of gestation, more than 90% of the spontaneously delivered pups died within the first 4 days of life. Litter size was significantly reduced relative to controls. Daily trioxane administration at 580 mg/kg body weight for the same period of time depressed maternal body weight gain but did not affect litter size or the development of the offspring. However, the pups showed significantly reduced motor activity relative to the controls at the age of 8 weeks. The active avoidance acquisition test revealed a significant decrease in responsiveness at the age of 18 and 19 weeks. Administration of trioxane at 190 mg/kg body weight was devoid of any effect in both the mothers and the pups in this study. Seven-week oral treatment of female rats (5 days/week) with trioxane at 1160 mg/kg body weight resulted in significant prolongation of the oestrus cycle, mainly of the dioestrus phase, which was reversible during the post-exposure observation period of 4 to 5 weeks. The animals showed marked clinical signs of toxicity while under treatment. Dose levels of 190 and 580 mg/kg body weight had no effect on the oestrus cycle.

Two very inadequately documented reports indicate that trioxane may have a skin-sensitising potential in humans.

Trioxane has been legally classified in the TRGS 905 and placed into category R_D3 of substances toxic to reproduction (i.e. “substances which cause concern for humans owing to possible developmental toxic effects”) in accordance with the EU classification criteria.

2 Name of substance

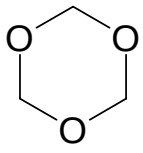
2.1	Usual name	Trioxane
2.2	IUPAC name	1,3,5-Trioxacyclohexane
2.3	CAS No.	110-88-3
2.4	EINECS No.	203-812-5

3 Synonyms, common and trade names

Aldeform
Formaldehyde, cyclic trimer
Formaldehyde trimer
Marvosan
Metaformaldehyd
Metaformaldehyde
Triformol
1,3,5-Trioxacyclohexan
Trioxan
s-Trioxane
sym-Trioxane
1,3,5-Trioxane
Trioxymethylen
Trioxymethylene

4 Structural and molecular formulae

4.1 Structural formula



4.2 Molecular formula $C_3H_6O_3$

5 Physical and chemical properties

5.1 Molecular mass, g/mol 90.08

5.2	Melting point, °C	60.2 (Lide and Frederikse, 1997) 62–63 (Reuss et al., 2000) 62–64 (Hoechst, 1989 a) 64 (Budavari et al., 1996; Sax, 1999)
5.3	Boiling point, °C	114.5 (Budavari et al., 1996; Sax, 1999; Lide and Frederikse, 1997) 115 (Hoechst, 1989 a; Reuss et al., 2000)
5.4	Vapour pressure, hPa	16.9 (at 25 °C) (Reuss et al., 2000) 17.3 (at 25 °C) (Hoechst, 1989 a; Sax, 1999) 41.6 (at 37.5 °C) 377 (at 86 °C) 440 (at 90 °C) (Reuss et al., 2000)
5.5	Density, g/cm³	1.17 (at 65 °C) (Budavari et al., 1996; Lide and Frederikse, 1997; Sax, 1999; Reuss et al., 2000) 1.39 (as crystals) (Reuss et al., 2000)
5.6	Solubility in water	Very soluble (Lide and Frederikse, 1997; Sax, 1999) 172 g/l (at 18 °C) 211 g/l (at 25 °C) (Budavari et al., 1996; Reuss et al., 2000) 267 g/l (at 25 °C) (Hoechst, 1989 a) Completely soluble (at 100 °C) (Reuss et al., 2000)
5.7	Solubility in organic solvents	Soluble in ethanol, ether and benzene (Lide and Frederikse, 1997) Easily soluble in alcohols, ketones, ethers, acetone, chlorinated hydrocarbons and aromatic hydrocarbons; only slightly soluble in pentane, petroleum ether and lower paraffins (Budavari et al., 1996; Sax, 1999) Soluble in alcohol, ketones, esters, organic acids, ethers, phenols, chlorinated hydrocarbons and aromatic hydrocarbons; only sparingly soluble in aliphatic hydrocarbons (Reuss et al., 2000)

5.8	Solubility in fat	No information available
5.9	pH value	No information available
5.10	Conversion factor	1 ml/m³ (ppm) ≙ 3.68 mg/m³ 1 mg/m³ ≙ 0.27 ml/m³ (ppm) (at 1013 hPa and 25 °C)

6 Uses

Primarily used in the production of (polyoxymethylene (POM)) plastics and as a formaldehyde-releasing agent, because it depolymerises under appropriate conditions to yield – very pure – formaldehyde; used e.g. as a textile auxiliary, in cross-linking agents in the manufacturing of carbon fibres, for the treatment of wood to produce musical instruments and in the ceramic industry. The chemical is also used as a stabilising agent, technical deodorant and anticorrosive additive, as an additive in photographic developers, as a solid fuel and in coating removers and disinfectants (Reuss et al., 2000).

7 Experimental results

7.1 Toxicokinetics and metabolism

The results from single-dose and multiple-dose studies indicate that trioxane is absorbed by the body upon oral administration or inhalation exposure. There is no experimental evidence that the chemical is absorbed via the skin.

Three male and 3 female rats (Sprague-Dawley, weighing 221 to 246 g) were treated with a single trioxane dose of 2500 mg/kg body weight, administered by oral gavage. The trioxane was radioactively labelled with ^{14}C (no adequate details given as to specific radioactivity or amount of radioactivity administered). The animals were housed in metabolism cages. The amounts of radioactivity excreted in the urine and faeces and eliminated as CO_2 in the expired air were determined after 12, 24, 48 and 72 hours. At 24 hours, the fractions of administered radioactivity excreted by males and females were 63.3% and 58.5%, respectively. At 48 hours, excretion was practically complete. By 72 hours after treatment, males had excreted a

total of 90% of the radioactivity, with 73.8% being recovered as CO_2 in the expired air, 15.4% in the urine and 0.8% in the faeces. Over the same period, females excreted 85.2% of the radioactivity, with 71.5% being recovered as CO_2 in the expired air, 13% in the urine and 0.7% in the faeces. The recovery of radioactivity in tissues from males and females was only 1.8% of the administered amount. Hence, there were no sex differences in absorption, elimination and excretion of trioxane in rats (Bio/dynamics, 1980 a).

The tissue distribution and binding of trioxane were investigated in pregnant 3.5-month-old rats. A single dose of 40 mg/kg body weight (1.6 MBq/kg body weight) of ^{14}C-labelled trioxane (specific radioactivity given as 3.6 MBq/mmol), dissolved in physiological saline solution, was administered by gavage to 3 pregnant females on day 19 of gestation. At 1, 2, 3, 5, 24, 27 and 48 hours after dosing, 30 µl samples of venous blood were obtained and analysed for content of radioactivity. The highest percentage, 4.67%, of total radioactivity administered was found at 5 hours. At 48 hours, the value was down to 0.6%. Plasma half-life was graphically determined as being approximately 14.5 hours. In further experiments, groups of 3 pregnant females were treated with the same oral dose of ^{14}C-labelled trioxane on day 19 of gestation and killed at 3, 24 or 48 hours after dosing. The liver, kidneys, lungs, brain, spleen, heart and a piece of fat were obtained from the maternal animals, as were the liver, kidneys, brain, a fragment of skin and the carcasses of the foetuses. The tissues were homogenised where necessary and analysed for radioactivity. The females' placentas and amniotic fluid were studied in the same manner. In order to investigate the tissue binding of radioactivity, homogenates were precipitated with 10-percent trichloroacetic acid and washed several times with 70-percent alcohol prior to determination of radioactivity in the residue. In all tissue and blood plasma samples obtained from the animals, the respective total recoveries at 3, 24 and 48 hours were 9, 7.4 and 4.2% of the administered radioactivity, with by far the largest fractions being found in the liver (3.3, 2.9 and 1.8%), the blood plasma (2.3, 1.5 and 0.6%) and the whole foetuses (0.38, 0.29 and 0.16%) at these three scheduled sacrifices. The other organs which were examined, with the exception of the kidneys, contained only very small fractions of the radioactivity. Most tissues and body fluids showed rapid elimination of radioactivity so that levels measured at 24 hours were markedly lower than those found at 3 hours. Elimi-

nation was fastest from maternal brain and amniotic fluid, with the respective half-lives of elimination being determined as 8 and 9 hours. However, radioactivity accumulated continually over time in some organs, particularly foetal liver and kidneys, so that levels of radioactivity detected in those tissues were markedly higher at 48 hours than they were at 3 hours after dosing. Quantification of the proportion of radioactivity that was firmly bound to tissue demonstrated that the contribution of tissue-bound activity to the total amount of recovered radioactivity increased over time. The proportion was lowest in the brain of pregnant females and their foetuses 3 hours after treatment with trioxane. The percentage of firmly bound radioactivity was higher in foetal brain and kidneys than it was in the corresponding organs of pregnant females. Tissue/plasma coefficients for trioxane were established for the major organs on the basis of the distribution of radioactivity. Three hours after administration, concentration levels of trioxane which were higher than those observed in plasma were found only in the livers of pregnant females; at 24 hours, levels higher than plasma levels were also detected in foetal liver and kidneys; and at 48 hours, finally, levels lower than plasma levels were found only in the brain, heart, muscle and fat of pregnant females and in the amniotic fluid. According to the investigators' conclusions from their overall evaluation of the results, the data suggested that after repeated administration to pregnant rats, retention of trioxane and/or one of its metabolites in the liver, kidneys and brain of foetuses could be markedly higher than in the corresponding organs of the mothers (Sitarek et al., 1990).

In a further study investigating the distribution, excretion and metabolism of trioxane, two groups of 42 male rats (Wistar, mean body weight 220 g) were administered ^{14}C-labelled trioxane (radioactivity/animal being given as 870 kBq) as single intraperitoneal doses of 40 or 400 mg/kg body weight. The animals were subsequently placed in metabolism cages, and urine and faeces were collected over 24-hour periods for 72 hours. The exhaled air containing expired CO_2 and unchanged trioxane was collected at hourly intervals during the first 14 hours after dosing, and at 24 hours, and all excreta were analysed for radioactivity. At intervals, 6 rats/dose each had 30 µl blood samples drawn from the tail vein, and the plasma and red blood cells were separately analysed for radioactivity. Finally, subgroups of 6 animals from each dose group were killed at 2, 8, 12, 24, 48 or 72 hours after dosing. The liver, kidneys, lung, brain, spleen and a section of fat tis-

sue and the sciatic nerve were removed and analysed for content of radioactivity. Following administration at 40 mg/kg body weight, ^{14}C-trioxane was excreted almost completely (87% of the administered radioactivity) in the form of CO_2 over the first 24 hours. The urine collected over the same period of time contained only 2.2% of the administered radioactivity, while the faeces contained only traces of radioactivity. Total excretion over a period of 72 hours amounted to 90%. The high trioxane dose of 400 mg/kg body weight was associated with initially increased excretion of radioactive CO_2 up to 12 hours after dosing. Within 24 hours, 70% of the administered radioactivity was excreted as CO_2. In addition, excretion of unchanged trioxane occurred primarily during the first hour after administration, amounting to 8% of the administered radioactivity at 24 hours after dosing. During this period of time, less radioactivity was excreted in the urine (1%) but slightly more in the faeces (1.3% after 72 hours). Total excretion after 72 hours was 80% of the administered radioactivity. The levels of radioactivity measured in the blood corresponded to the time-course of excretion. Following administration of ^{14}C-trioxane at 40 mg/kg body weight, there was a rapid decline again in blood radioactivity levels, which was consistent with an open two-compartment system with half-lives of 4.45 and 69.3 hours. Erythrocytes bound only minute amounts of radioactivity. At the higher ^{14}C-trioxane dose level of 400 mg/kg body weight, the amount of radioactivity in erythrocytes was 10 times higher than in plasma. Levels in both samples increased during the first few hours but then declined rapidly after 4 to 6 hours. Again, decline was biphasic with half-lives of 6.8 and 55.4 hours for plasma and 1.7 and 32.8 hours for erythrocytes. The amounts of radioactivity detected in the examined tissues and plasma were small compared with the amount administered. When ^{14}C-trioxane was administered at 40 mg/kg body weight, most of the radioactivity appeared in the liver after 2 hours, while the lowest levels were found in the fat tissue, the sciatic nerve and the brain. There was a marked decline in radioactivity with time in all tissues and in the plasma. At 72 hours, only 1.2% of the administered radioactivity was found in the body. When animals were treated with ^{14}C-trioxane at 400 mg/kg body weight, the highest levels of radioactivity were observed in plasma and in the liver and kidneys. Levels in the plasma and liver rose during the first 8 to 12 hours and then showed some decline, as noted in the other tissues. Overall, the amount of radioactivity remaining in the body was, again, small compared with the administered amount and did not exceed 1.5% of the administered dose. The investigations demon-

strated that trioxane underwent rapid absorption and distribution in the blood and organs upon intraperitoneal administration. The compound was almost completely metabolised and excreted as CO_2 in the expired air. The investigators suspected that trioxane was hydrolysed to formaldehyde, which subsequently underwent oxidation to formic acid and ultimately CO_2. Excretion was rapid, and there were no indications that trioxane itself or any metabolite or transformation product accumulated in the body (Ligocka et al., 1998).

In order to investigate the biotransformation of trioxane, the chemical was added to whole blood from rats at levels of 10, 100 or 500 µl/ml and incubated at 37 °C for one hour. The respective average recoveries for trioxane were 73, 93 and 80% (as determined by extraction with methylene chloride and subsequent analysis by gas chromatography). The investigators interpreted their results as suggesting that little, if any, metabolism of trioxane to formic acid and/or formaldehyde occurred in blood. Despite considerable analytical efforts, determination of the two proposed metabolites failed due to methodological problems (Bio/dynamics, 1980 b).

7.2 Acute and subacute toxicity

The acute toxicity data for trioxane are summarised in Table 1.

Beginning of Table 1

Table 1. Acute toxicity of trioxane following single-dose administration

Species, strain, sex*	Route	Dose (mg/kg body weight)	Effects	Observation period	Reference
Rat	oral	9500	LD_{50} (survival 24 to 48 hours); narcotic effect	no data	Hoechst, 1963
Rat	oral	8190	LD_{50}	14 days	Rinehart et al., 1967
Rat	oral	> 3200	LD_{50}; no mortality; dyspnoea, apathy; no findings at necropsy	7 days	BASF, 1968
Rat, Wistar, male	oral	8500	LD_{50}; narcotic effect	no data	Czajkowska et al., 1987; Indulski et al., 1986
Rat, Wistar, female	oral	7740	LD_{50}	no data	Sitarek et al., 1988
Rabbit	dermal	> 3980	LD_{50}; no mortality; slight to moderate erythema which was reversible within the observation period	14 days	Rinehart et al., 1967

Table 1. Acute toxicity of trioxane following single-dose administration					
Species, strain, sex*	Route	Dose (mg/kg body weight)	Effects	Observation period	Reference
Rabbit, White Vienna	dermal	> 15000	LD_{50}; narcotic effect, no skin effects	no data	Czajkowska et al., 1987; Indulski et al., 1986
Rat	inhalation	atmosphere saturated at 20 or 70 °C, 8 hours	no mortality after exposure to atmosphere saturated at 20 °C; atonia, reeling and tremor; no findings at necropsy; mortality was 4/6 after exposure to atmosphere saturated at 70 °C; dyspnoea, mucous membrane irritation, reeling, encrusted snouts in several cases and hyperaemia of the lungs	no data	BASF, 1968
Rat, Wistar, male	inhalation	> 26000 mg/m³, 4-hour exposure	LC_{50}; no mortality; retardation of body weight gain	14 days	Czajkowska et al., 1987; Indulski et al., 1986
Rat	inhalation	> 39100 mg/m³, 4-hour exposure	LC_{50}; no mortality; reduction in body weight gain, respiratory distress	14 days	Celanese, 1986
Rat	intraperitoneal	850	LD_{50}	no data	Jaros-Kaminska et al., 1985
Mouse	intraperitoneal	ca. 1800	LD_{50}; dyspnoea, saltatory spasms, apathy, late deaths, adhesions and residual test material in the abdominal cavity	7 days	BASF, 1968
* where indicated					

End of Table 1

According to the reported data, the LD_{50} values for oral administration to rats were between 7740 and 9500 mg/kg body weight. The LD_{50} values for dermal exposure of rabbits were reported as > 3980 and > 15000 mg/kg body weight. Inhalation exposure of animals to concentration levels of up to 39100 mg/m³ also caused no marked acute toxicity under normal conditions. However, inhalation of a trioxane vapour/air mixture saturated at 70 °C resulted in the death of 4 out of 6 exposed rats after 8 hours. Based on all the data available, the chemical is to be considered as being of very low acute toxicity.

Oral administration of very high trioxane doses to rats produced rather unspecific signs of toxicity in addition to a narcotic effect, which reportedly also occurred in rabbits upon dermal application of trioxane at 15000 mg/kg body weight. LD_{50} values found in rats and mice after intraperitoneal administration of trioxane were 850 and 1800 mg/kg body weight, on the whole suggesting low toxicity despite the great difference between the values.

Three male and 3 female rats (Sprague-Dawley, weighing 221 to 246 g) were treated with a single oral dose of trioxane at 2500 mg/kg body weight. The animals appeared very inactive after dosing and had poor motorability. One rat had very laboured and rapid breathing. The clinical signs were only seen during the first 24 hours. The animals, which were sacrificed 72 hours after dosing, showed no treatment-related effects at necropsy (Bio/dynamics, 1980 a).

In a subsequent 4-week study conducted in accordance with OECD guideline No. 407, groups of 5 male and 5 female Wistar rats (Hoe:WISKf, SPF71, approx. 6 weeks old at the beginning of the study) each received daily doses (7 times per week) of an aqueous solution of trioxane (99.1% pure) at 40, 200 or 1000 mg/kg body weight by oral gavage. The behaviour and the general physical condition of the rats were normal. Body weight gain was unaffected even at the highest dose level, as was food and water consumption. There were no deaths. Haematological examination at the end of the treatment period revealed statistically significant reductions in leukocyte counts in the male and female rats treated at 1000 mg/kg body weight. The clinical chemistry findings noted in the top dose group included slightly elevated levels of γ-glutamyl transpeptidase activity in male and female rats as well as increased levels of glutamic-pyruvic transaminase and glutamic-oxaloacetic transaminase and decreased protein and glucose levels in the females from that group. Autopsy revealed no remarkable substance-related changes, neither did microscopic examination. The *no effect level* was 200 mg/kg body weight (Hoechst, 1990).

Groups of 5 male and 5 female Sprague-Dawley rats (respective mean initial weights 204 and 199 g) inhaled trioxane at concentration levels of 0 (controls), 100, 1000 or 5000 ppm (equivalent to 0, 368, 3680 and 18400 mg/m³) for 6 hours per day, 5 times per week for 2 weeks (10 exposures). The mean analytical concentrations were 103, 984 and 4940 ppm (equivalent to 379, 3621 and 18179 mg/m³). There were no deaths. The rats of the 5000 ppm group exhibited a reduced righting reflex and persistent pupillary constriction, and body weight values were reduced throughout most of the study. Haemoglobin, haematocrit and erythrocyte and lymphocyte counts were increased, while total leukocyte count and segmented neutrophils were decreased. Serum glutamic-pyruvic transaminase activity, total protein and albumin were slightly to significantly increased, while blood glucose was decreased. In the absence of supportive histopathological find-

ings, the toxicological significance of these findings was considered unclear by the investigators. Relative spleen weights for the animals from the top dose group were decreased. Histopathology revealed squamous metaplasia of the mucosa of the anterior nasal cavity, the epithelium showing necrosis and desquamation. Moreover, acute rhinitis was noted. In the 1000 ppm male and female rats and the 100 ppm male rats, the only treatment-related effect noted was a decrease in absolute and relative spleen weights (Bio/dynamics, 1983).

7.3 Skin and mucous membrane effects

The skin irritancy of trioxane was investigated in 3 female white New Zealand rabbits (initial weights ranging from 2.2 to 2.5 kg) in accordance with OECD guideline No. 404. The animals received a single 4-hour (semi-occlusive) application of 0.5 g of the chemical (99.1% pure), made into a paste with 0.2 ml of 0.9-percent saline solution. Effects were scored 30 to 60 minutes and 24, 48 and 72 hours upon removal of the chemical. None of the scheduled assessments yielded any signs of irritation (the average irritation scores for erythema and oedema were both 0.0). Trioxane thus proved not to be irritating to the skin in this study (Hoechst, 1989 b).

Trioxane (ground, applied on moistened patches) caused no irritation to the clipped skin of the rabbit after (semi-occlusive) exposure for 1, 5 or 15 minutes. Following 20-hour (occlusive) application, slight reddening and severe oedema occurred at 24 hours and severe circumscribed necrosis and severe scale formation were seen at 8 days (BASF, 1968).

The intact rabbit skin showed no reaction when 500 mg trioxane was applied for 24 hours. Assessment yielded a Draize score of 0 (no further details; Rinehart et al., 1967; Celanese, 1986).

Single application of trioxane to the skin of rabbits (White Vienna) produced no signs of irritation. Daily treatment with the chemical for 10 days resulted in mild irritation of the skin (no further details; Indulski et al., 1986).

Determination of the skin irritancy of trioxane according to the Draize method and repeated daily applications of the chemical demonstrated no effects (no further details; Czajkowska et al., 1987).

Three female New Zealand rabbits (with initial weights ranging from 2.3 to 2.7 kg) were each given a single instillation of 100 mg trioxane (99.1%

pure) into the conjunctival sac of the left eye in accordance with OECD guideline No. 405. After 24 and 48 hours, the eyes were rinsed out with physiological saline solution. The effects were assessed 1, 24, 48 and 72 hours after treatment. From 1 to 48 hours after treatment the animals exhibited diffuse crimson to diffuse red colouration of the conjunctivae and slight conjunctival swelling. In addition, clear colourless discharge was observed. In one animal, the cornea was slightly clouded and the details of the iris were slightly obscured one hour after treatment. At 24 hours, the iris was reddened. The signs of irritation were reversible 72 hours following instillation. The mean irritation indices for reddening of the conjunctiva, conjunctival swelling, iritis and clouding of the cornea were 1.7, 0.7, 0.1 and 0.0, respectively (Hoechst, 1989 c). The investigators evaluated the mucous membrane effects of trioxane as not requiring labelling on the basis of the above-mentioned results and the classification criteria set forth in Directive 83/467/EEC and the German Hazardous Substances Ordinance ("Gefahrstoffverordnung").

In an eye irritation study of trioxane in rabbits, the effects were evaluated as constituting mild irritation with scores of 17 out of 110 noted on the Draize scale after 24 hours. At 72 hours, the findings had not yet cleared up completely (no further details; Rinehart et al., 1967).

Single instillation of trioxane into the rabbit eye resulted in severe irritation (no further details; Indulski et al., 1986).

In a further eye irritation study in the rabbit, there was severe reddening and very severe oedema of the conjunctivae in addition to slight cloudiness of the cornea one hour after single instillation of 50 mm^3 of powdered trioxane. At 24 hours, there was some improvement in the oedema, but the other effects persisted. However, all of the signs had cleared up completely 8 days after treatment (BASF, 1968). Thus, trioxane was irritating to the eye in this study.

Twenty-four hours after 6 rabbits each had one eye treated with 100 mg trioxane, marked conjunctivitis was observed in all 6 exposed eyes. Iritis occurred in 3 cases, corneal opacities in 5 cases and corneal ulceration in 5 cases. All eyes were free of irritation within 10 days (no further details; Celanese, 1986). Thus, trioxane was severely irritating in this study.

Trioxane was tested in the rabbit eye according to the Draize method. The irritation score was 58 out of a maximum score of 110 points one day after

application (indicating severe irritation), but all of the effects had cleared up completely by 6 days after treatment (no further details; Czajkowska et al., 1987). Thus, trioxane was severely irritating to the eye in this study.

7.4 Sensitisation

A maximisation test which was carried out in the guinea pig in accordance with OECD guideline No. 406 showed that trioxane (99.9% pure, flaky product) had no sensitising effect. Intradermal induction was accomplished with a 5-percent aqueous solution and dermal induction with a 50-percent aqueous solution in 20 female guinea pigs (initial weights ranging from 259 to 319 g). Control groups of 10 animals were treated with trioxane either at both scheduled challenge times or only at the second scheduled challenge without prior induction. After both the first challenge (20 days after the intradermal induction) and the second challenge (27 days after the intradermal induction), which were both carried out with a 50-percent solution, none of the 20 animals treated showed a positive reaction (BASF, 1989).

A study on the skin-sensitising potential of trioxane was conducted in male guinea pigs (Hartley strain) weighing approximately 300 g. Ten animals each had four 0.1 ml aliquots of a trioxane solution (no details of solvent or concentration) applied to the clipped skin of the back within a period of 10 days. The concentration used was the highest concentration which had not caused primary irritation in a preliminary study. At the time of the third application, 0.2 ml Freund's adjuvant was injected intradermally at one point adjacent to the application site (split adjuvant test). After a 2-week rest period, the animals were challenged on the clipped flanks with trioxane solution on the one flank and the solvent on the other flank, which served as a control. After 24 and 48 hours, the animals were evaluated for oedema and erythema formation. Moderate skin changes of this type in 2 or more out of 10 animals were considered a positive result. In the case of trioxane, an effect was noted only in one animal, and therefore the chemical was evaluated as not sensitising to the skin (Rao et al., 1981).

In a maximisation test in guinea pigs (Hartley strain) no skin-sensitising potential was noted for trioxane (no further details; Indulski et al., 1986).

A study investigating the sensitising properties of trioxane by means of the Magnusson and Kligman method gave a negative result (no further details; Czajkowska et al., 1987).

7.5 Subchronic and chronic toxicity

Groups of 8 to 10 male Wistar rats (weighing 200 to 230 g) received trioxane by oral gavage at daily dose levels of 106 or 213 mg/kg body weight on 5 days/week for 7 months or 850 mg/kg body weight on 5 days/week for 4 months. The dosages corresponded to $^1/_{80}$, $^1/_{40}$ and $^1/_{10}$ of the LD_{50}. The tests and observations included: behaviour, monthly body weight determinations and mortality; erythrocyte, leukocyte, thrombocyte and reticulocyte counts, haematocrit, haemoglobin, differential blood count and clotting time; total protein, albumin, urea and bilirubin; and alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, sorbitol dehydrogenase and acetylcholine esterase activities. The heart, liver, lungs, spleen, adrenal glands and kidneys were weighed and histopathologically examined. Body weight gain was reduced by approx. 2.5 and 5% relative to controls in the animals of the 213 and 850 mg/kg groups, respectively, while it was approx. 15% higher than controls in the 106 mg/kg group. At 213 and 850 mg/kg body weight, trioxane produced irritation of the gastric mucosa. No cumulative effect of trioxane was observed with respect to mortality (no further details; Czajkowska and Krysiak, 1987; Indulski et al., 1986).

In an older, poorly documented subacute toxicity study, trioxane was administered to adult rats in their feed until they died after 16 to 67 days or were killed after 30 to 99 days. No details were given regarding the dose of trioxane administered or the concentration of trioxane in the feed. Out of the 14 rats used in the study, the longer surviving rats displayed hyperchromic anaemia and a severe erythroblastic reaction in the bone marrow (7 rats). All of the animals gained hardly any weight or had decreased body weights at the end of the study. The observed clinical signs resembled those seen after a protein-deficient diet (no further details; Piette, 1948).

Groups of male Wistar rats (weighing approx. 250 g) and groups of female Himalayan guinea pigs (weighing approx. 280 g) inhaled air containing trioxane levels of 0 (controls), 50, 500 or 2500 mg/m³ during 5 hours/day on 5 days/week for 12 months. In the rat, the following parameters were investigated: behaviour, body weight, food consumption, body temperature and mortality; every month, except at months 7 and 9: motor co-ordination and behaviour on a treadmill; at 3, 6 and 12 months: erythrocyte, leukocyte, thrombocyte and reticulocyte counts; haematocrit, haemoglobin, dif-

ferential blood count and clotting time; at 1, 3, 6 and 12 months: total protein, albumin, urea, glucose, inorganic phosphate, calcium, chloride; and alanine aminotransferase, aspartate aminotransferase, acetylcholine esterase, alkaline phosphatase and sorbitol dehydrogenase activities; and at 12 months: urinalysis (specific weight, pH, protein, glucose, ketones, bilirubin, blood, sediment). Following necropsy, organs (no further details) were weighed and examined by light microscopy. The liver and trachea were additionally examined by electron microscopy. In the guinea pigs, the respiratory rate and duration of inspiratory and expiratory phases were additionally determined every month except for months 7, 9 and 11 of exposure. In the rats, trioxane produced gradually increasing disturbances of motor coordination on the treadmill, the effect being most pronounced and statistically significant in all treatment groups after 12 months of exposure. However, there was no concentration-effect relationship. Serum acetylcholine esterase activity was reduced by approx. 30% in all concentration groups at the end of exposure. At 2500 mg/m³, trioxane produced histopathological changes of the kidneys and respiratory tract, whereas 500 and 2500 mg/m³ gave rise to epithelial lesions in the trachea (no further details). The 50 mg/m³ concentration showed no evident histopathological changes. The guinea pigs exhibited no changes in respiratory function during the treatment period. The trioxane concentration of 50 mg/m³ of air was given as the threshold concentration in rats (Indulski et al., 1986).

7.6 Genotoxicity

7.6.1 In vitro

The available data on the in-vitro genotoxicity of trioxane are summarised in Table 2.

Beginning of Table 2

Table 2. In-vitro genotoxicity tests with trioxane

Test system	Concentration range tested (µg/plate or µg/ml)*	Metabolic activation system	Result		Reference
			with metabolic activation	without metabolic activation	
1 Gene mutation tests in bacteria					
Escherichia coli Sd-4-73, induction of streptomycin-independent revertants	"a small crystal"	not used	not tested	negative	Szybalski, 1958

Table 2. In-vitro genotoxicity tests with trioxane					
Test system	Concentration range tested (µg/plate or µg/ml)*	Metabolic activation system	Result		Reference
			with metabolic activation	without metabolic activation	
Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538, standard plate incorporation test	0.5–5000 (no bacteriotoxicity)	S-9 mix from Aroclor-induced rat liver	negative	negative	Litton Bionetics, 1980 a
Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538, standard plate incorporation test	0.5–5000 (no bacteriotoxicity)	S-9 mix from Aroclor-induced rat liver	negative	negative	Kowalski et al., 1984
Salmonella typhimurium TA 97, TA 98, TA 100, TA 1535, TA 1537 preincubation test	10000 (98% pure)	S-9 mix from Aroclor-induced rat liver	negative	negative	Zeiger et al., 1988
Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, standard plate incorporation test and preincubation test	20–5000 (no bacteriotoxicity, 99% pure)	S-9 mix from Aroclor-induced rat liver	negative in both tests	negative in both tests	BASF, 1988
2 Gene mutation tests in mammalian cells					
Mouse lymphoma cells L5178Y/TK+/-, trifluorothymidine resistance assay	313–15000 mg/ml without S-9 mix (low cytotoxicity), 156–7500 mg/ml with S-9 mix (high cytotoxicity at and above 625 mg/ml)	S-9 mix from Aroclor-induced rat liver	positive	negative	Litton Bionetics, 1980 b
V79 cells of the Chinese hamster, HPRT test, 6-thioguanine resistance assay	100–900 (99.9% pure, no cytotoxicity)	S-9 mix from Aroclor-induced rat liver	negative	negative	Hoechst, 1992 a
V79 cells of the Chinese hamster, chromosome aberration test	90.0–909 (99.9% pure, no cytotoxicity)	S-9 mix from Aroclor-induced rat liver	negative	negative	Hoechst, 1992 b
* Unless stated, publications give no data on the cytotoxic effects or the purity of the trioxane used.					

End of Table 2

The available in-vitro genotoxicity studies were almost all negative. Five tests employing bacteria produced only negative results. The same was found for V79 cells of the Chinese hamster when investigated in an HPRT test and a chromosome aberration test. Only a test in mouse lymphoma cells yielded a positive result when carried out in the presence of a metabolic activation system. However, the outcome of the parallel test which was run without metabolic activation was negative. Both tests were carried out using very high trioxane concentrations, which resulted in severe cytotoxicity in the case of the test with the positive result in the presence of

metabolic activation. The severity of the mutagenic effect was directly linked to the cytotoxicity so that the relevance of that result must be questioned.

7.6.2 In vivo

In a micronucleus test, male BALB/c mice (7 to 8 weeks old) received trioxane at 2125 or 4250 mg/kg body weight in two intraperitoneally administered doses. Four animals were used per dose group and control. Six hours after treatment, bone marrow preparations were obtained from the femur. A total of 8000 polychromatic erythrocytes were scored in the low dose group and the control group, while 7550 were scored in the high dose group. There was no increase in micronucleated polychromatic erythrocytes as compared with the control (Przybojewska et al., 1984).

A dominant-lethal test was carried out in male Wistar rats, which received trioxane at 850 or 1700 mg/kg body weight/day as an aqueous solution by oral gavage on 5 days/week for 8 weeks. The doses were equivalent to 10 and 20% of the LD_{50}, respectively. Ten animals were used per dose or control group. The controls received the vehicle, water. Males were mated with untreated females by 1 : 2 pairing every week during the 8-week treatment period. Necropsy of the female rats was performed 13 or 14 days after the middle of the mating interval. For the trioxane-treated groups, the fertility index and the numbers of live and dead foetuses were in the normal range. No substance-related dominant lethal factors were observed. Some males were found to have focal necrosis of the seminiferous epithelium and alteration of spermatogenesis (no further details; Baranski et al., 1984).

In a further dominant-lethal test, male Wistar rats inhaled air containing trioxane at 2500 mg/m³ in a dynamic inhalation chamber for 5 hours/day on 5 days/week for 12 months. Fourteen animals were used per dose or control group. At the end of the study, the males were mated with untreated females by 1 : 2 pairing. Necropsy of the females was performed 13 or 14 days after the middle of the mating interval. For the trioxane-treated group, the fertility index and the numbers of live and dead foetuses were in the normal range. Neither substance-related dominant lethal factors nor preimplantation losses were observed (Baranski et al., 1984).

Inhalation exposure of *Drosophila melanogaster* to trioxane led to a slight increase in sex-linked recessive lethal mutations from 0.18% in the control

group to 1.02%. For the purposes of the study, males were placed in a closed glass vessel and exposed to air containing trioxane at 66.7 mg/l for 12 days (no further details of the methodology used). Evaluation was based on 683 F_1 pairs (controls: 4431 pairs). Dietary administration of 10 to 25 mg of trioxane for 2 to 8 days caused no increase in recessive lethal mutations in the male larvae or the adult males. The respective numbers of F_1 pairs examined were 763 and 917 (controls: 5305 and 5942 pairs; Filippova et al., 1967).

In order to determine the DNA damage induced in the hepatocytes of trioxane-treated animals, rats weighing 150 to 200 g were administered single doses of 425 or 850 mg/kg body weight (equivalent to one-half the LD_{50} and the LD_{50}, respectively) by intraperitoneal injection. Four hours later, the animals were killed, the livers excised and the number of single-strand breaks in the hepatocytes ascertained by means of the alkaline elution method. The elution rate constant K and the DNA fragmentation index (DFI) were used as measures of DNA damage. For both dosages, K was significantly higher than in untreated control animals. However, no dose-dependence was observed. Both dosages resulted in an increase by about 80%. In contrast, the DNA fragmentation index only had a very small value of 0.001 to 0.002 as compared with the concurrent mitomycin C control, in which K was increased by 160% and the DFI was 2.56. The investigators assumed that trioxane is able to affect the physico-chemical properties of DNA in hepatocytes and may thus create the risk of a mutagen effect (Jaros-Kaminska et al., 1985).

In accordance with the OECD guidelines for testing, a UDS test was carried out in which DNA repair in hepatocytes was measured by determination of ^{3}H-thymidine incorporation. Groups of 6 male Wistar rats (Chbb:THOM) with a mean weight of 258 g were administered single oral doses of trioxane (99.9% pure) at 250, 500, 1000 or 2000 mg/kg body weight, as a solution in water. Three animals/group were sacrificed 4 and 18 hours later and hepatocytes were isolated from the livers. The cells were subsequently incubated with ^{3}H-thymidine, and DNA repair was measured as incorporated radioactivity after 18 hours. No increase in radioactivity was detected in hepatocytes from treated rats as compared with controls, and hence the result of the test was negative (BASF, 1997).

7.7 Carcinogenicity

No results are available from carcinogenicity studies in experimental animals. In vitro, a cell transformation test was carried out using C3H 10T-1/2 cell cultures (mouse embryo cells). Cultures of 300 cells were incubated with trioxane concentrations of 0 (control), 1, 10, 100, 500, 1000, 5000, 10000 or 20000 µg/ml for 24 hours and subsequently transferred to, and maintained in, normal medium. Cytotoxicity was measured up to a concentration of 10000 µg/ml where it reached 48%. No colony formation occurred at 20000 µg/ml. Eleven days upon incubation with trioxane the medium was removed from 6 cultures/concentration, and the cell layers were washed, fixed and stained. The colonies which had formed were examined for transformations. The remaining 6 cultures/concentration were maintained in medium for another 27 days and then processed and scored in the same manner as the other cultures. No evidence of transformation was observed for either the cell colonies which were fixed at 11 days or the cell colonies which were cultured for 38 days whereas transformation occurred at the expected frequencies in the benzo(a)pyrene-treated positive controls at both time points of investigation (Environmental Pathology Laboratory, 1981).

7.8 Reproductive toxicity

The teratogenic potential of trioxane was investigated in a study conducted in accordance with OECD guideline No. 414 and the relevant EU Directive. Groups of 23 pregnant female rats (Wistar, 8 to 10 weeks old) were treated daily by oral gavage with an aqueous solution of trioxane at dose levels of 0 (controls), 100, 315 or 1000 mg/kg body weight on days 7 to 20 of gestation. On day 21 of gestation, the dams were sacrificed and necropsied. For all females, the gravid uterus was weighed, and the foetuses were removed, the number of live and dead foetuses, resorptions and corpora lutea determined and all specimens examined macroscopically. Half of the foetuses from each litter were fixed in alcohol, dissected and examined for anomalies of the internal organs. Subsequently, the skeletons were stained and examined under the stereo-microscope. The remaining foetuses were placed in Bouin’s solution and checked for organ anomalies. Treatment with trioxane caused no mortality or clinical signs of toxicity in the dams. The highest dose group exhibited slight but statistically significant reduction in body weight gain and food consumption from day 10 of gestation until

the end of the study. Corrected body weight gain (body weight on day 21 of gestation minus body weight on day 7 of gestation minus gravid uterus weight) was reduced at all dose levels, but particularly at the top dose level. Mean values for corrected body weight were 33.60 g in the control group, 27.13 g in the low dose group, 28.96 g in the intermediate dose group and 18.88 g in the high dose group. At necropsy and uterus weight determination, the treated animals showed no differences as compared with the controls. Litter size and the sex ratio within litters were also not affected by trioxane. In the top dose group, foetal body weight and length were decreased, whilst placental weight was increased. The top group had significantly more retarded foetuses. The frequency of resorptions was not affected by the administration of trioxane. In the top dose group, 5 dead foetuses were observed in 5 litters. Morphological examination of the foetuses from the top dose group revealed two cases of aplasia of the tail accompanied by aplasia of the sacral vertebral arches and centres and the caudal vertebral centres. The incidence of foetuses with bone defects was increased in this dose group. In addition, retarded ossification was observed in various bones. The examined foetuses from the intermediate dose group also showed increased incidences of wavy or thickened ribs and retarded ossification. The lowest dose was devoid of any effect on foetal morphology. The investigators gave 100 mg/kg body weight as the *no observed effect level* (NOEL) for the embryotoxicity and foetotoxicity of trioxane. With regard to maternal toxicity, a NOEL was not given due to the finding that corrected body weight was reduced in all dose groups (Hoechst Marion Roussel, 1998).

In a study designed to investigate the effect of trioxane on fertility in addition to the chemical's ability to induce dominant-lethal mutations (see also Section 7.6.2), male rats were given daily orally administrations of trioxane at 0 (control), 850 or 1700 mg/kg body weight on 5 days/week for 8 weeks. During every week of treatment, each male was mated with two females. A further study was carried out in which males inhaled a trioxane concentration of 2500 mg/m³ for 5 hours/day on 5 days/week for 12 months. They were subsequently each mated with 2 females for one week. Necropsy of the pregnant females was performed 13 or 14 days after the middle of the mating interval. No increase was observed in the numbers of preimplantation losses, dead implants and live foetuses/female in the treated pairs, as compared with the controls. Trioxane had no effect on the fertility of the

males, although microscopic examination of the testes of some treated males revealed focal necrosis of the seminiferous tubular epithelium and alteration of spermatogenesis (no further details; Baranski et al., 1984).

In order to investigate the prenatal toxicity of trioxane, groups of 16 to 22 3-month-old pregnant Wistar rats (weighing 205 to 245 g) were treated by oral gavage with doses of 0 (control), 770, 1550 or 3870 mg/kg body weight (equivalent to $^1/_{10}$, $^1/_5$ and $^1/_2$ of the previously determined LD_{50}), given as a 15-percent aqueous solution on alternate days from day 8 to day 20 of gestation. In a second series, groups of 13 to 17 pregnant Wistar rats were treated daily by oral gavage with an aqueous solution of trioxane (containing 0.001% formaldehyde) at dose levels of 0 (controls) or 190 mg/kg body weight or with an aqueous 0.5-percent solution of formaldehyde at 20 mg/kg body weight from day 8 to day 20 of gestation. Maternal weight gain and food and water intake were determined for all females, and the animals were sacrificed on day 21 of gestation. Upon necropsy, various maternal organs, including the placentas, were weighed and histopathologically examined. Embryotoxicity, foetotoxicity and teratogenic effects on the foetuses were assessed. No animals died during the study. The maternal animals treated at the two highest trioxane dose levels exhibited reduced body weight gain and daily food consumption, as compared with the controls. The group of animals treated with trioxane at 3870 mg/kg body weight had reduced absolute liver and placental weights and increased relative kidney and adrenal weights. Relative kidney weight was also increased at the 770 mg/kg dose level. Histopathological examination revealed an increase in the rate of hepatocellular mitosis and hydropic liver degeneration in the animals from the highest dose group, an increase in the rate of hepatocellular mitosis also being noted in the intermediate dose group. A proportion of the placentas of dams from all dose groups and control groups displayed histopathological changes (fibrin deposits, inflammatory infiltrations and focal necrosis). Compared with the controls, a markedly increased number of females with changes of the placenta was seen in the intermediate dose group, which was treated with 1550 mg/kg body weight (37/55, control: 26/81). At 190 mg/kg body weight, the number of females with changes of the placenta was increased (21 out of 87) relative to the comparatively low incidence of 7 out of 79 examined placentas seen in the control group used for comparison as opposed to the control group which was run concurrently with the high dose groups and in which

26 out of 81 placentas were affected. No other indications of trioxane-induced maternal toxicity were observed in animals treated with the chemical at dose levels of 770 or 190 mg/kg body weight. At and above the dose of 770 mg/kg body weight the number of resorptions increased dose-dependently and there was also a dose-dependent reduction in foetal body weight and length. The two higher doses of trioxane reduced the mean number of live foetuses per litter. Due to high intrauterine mortality and extremely small body size of the surviving foetuses at the highest dose it was not possible to study malformations at that dose. The dose levels of 770 and 1550 mg/kg body weight gave rise to malformations of the brain, the kidneys and/or the skeletal system and significantly increased the number of foetuses with delayed ossifications. No such effects were observed at a trioxane dose level of 190 mg/kg body weight or a formaldehyde dose level of 20 mg/kg body weight. The investigators concluded that in rats sufficiently high dose levels of trioxane resulted in foetal lethality, retarded foetal growth and congenital malformations. These effects occurred not only at maternally toxic doses, but also at 770 mg/kg body weight. The potential contribution of formaldehyde to the observed effects was excluded. However, the value of this study is limited by the fact that a number of females from all treatment and control groups were found to have histopathological changes of the placenta, the influence of which on the emergence of the observed foetotoxic effects is unclear (Sitarek et al., 1988).

In order to determine the effect of trioxane on the measured oestrus cycle of the rat, groups of 4-month-old female rats (mean weight 214 g) were treated with aqueous solutions of trioxane by oral gavage on 5 days/week for 7 weeks. Dose levels of 0 (controls), 190 and 580 mg/kg body weight were administered. Vaginal smears were taken daily during the first 14 days of treatment, during treatment weeks 6 and 7 and during weeks 4 and 5 following the end of treatment. The two lower dose levels had no effect on the length of the cycle or the duration of its individual phases. Animals treated at 1160 mg trioxane/kg body weight showed a significant increase in the length of the oestrus cycle at weeks 6 and 7 relative to the controls, mainly due to prolongation of the dioestrus phase. These animals also displayed overt clinical signs of toxicity, such as ruffled coat and discharge from the nose, and their body weight gain was significantly depressed relative to controls. All effects observed were reversible during the post-exposure observation period. The investigators concluded that trioxane

had no effect on the oestrus cycle since alterations of the oestrous cycle appeared only in conjunction with other severe signs of trioxane intoxication (Sitarek and Baranski, 1990 a).

A further study on the reproductive toxicity of trioxane investigated the postnatal development of the offspring of rats treated with the chemical during pregnancy. Groups of 14 to 17 pregnant Wistar rats (4 months old, weighing 190 to 210 g) were treated with trioxane from day 2 to day 20 of gestation. Trioxane was administered in aqueous solution by oral gavage either at 190 or 1160 mg/kg body weight every other day or at 580 mg/kg body weight daily. Two control groups received the equivalent volume of water, one every other day and the other every day. The mothers were allowed to nurse their progeny until they were 28 days of age. The number of live and dead pups and body weight at birth were recorded, as was body weight gain of offspring up to the age of 19 weeks. In addition, the neonates' morphological development was monitored (age at which eye opening and incisor eruption occurred). At the age of 4 and 8 weeks the number of erythrocytes, the haematocrit and the haemoglobin level were measured in the offspring's blood. Furthermore, neurodevelopmental and behavioural assessments were performed on the pups during the first 2½ weeks. Randomised groups of 10 pups/treatment group were assessed for motor activity at the age of 8, 11 and 14 weeks and for active avoidance acquisition throughout weeks 18 and 19. None of the dams died or showed clinical signs of toxicity while under treatment with trioxane. Trioxane treatment at 580 or 1160 mg/kg body weight caused marked depression of body weight gain. The dams treated at the highest dose level exhibited a statistically significant reduction in litter size. Over 90% of the pups that were born died, in most cases within the first 4 days of life, and hence no further assessments were carried out at that dose level. The two lower dose groups showed no effects on litter size or development of pups. Erythrocyte count, haematocrit and haemoglobin level remained unchanged as compared with the control. At 580 mg/kg body weight trioxane reduced motor activity of male pups aged 8 weeks to 70% of the control and also caused a statistically significant decrease in motor activity in female pups aged 8 and 14 weeks. When tested for active avoidance acquisition these animals showed a significant decrease in responsiveness. These effects were not observed in the group treated with 190 mg/kg body weight (Sitarek and Baranski, 1990 b).

7.9 Effects on the immune system

No information available.

7.10 Neurotoxicity

No information available.

7.11 Other effects

No information available.

8 Experience in humans

A total of 84 dentists, dental technicians and dental nurses with allergic contact dermatitis were patch-tested with the standard series of the CMEA countries and a number of occupational allergens. Trioxane (5 percent in petrolatum) was also included in the study as a disinfectant used in the dental area. Thirty-one (36.9%) of the patients had occupational allergic contact dermatitis. Among them there was one case of trioxane allergy (1.2% of all patients studied; Berova et al., 1990).

A very inadequately documented older study was carried out in 66 dental students (24 males, 42 females) in order to investigate the extent to which they developed allergies to 12 chemicals, among them trioxane, to which they had frequent exposure during their dental studies over a period of 3½ years. The subjects underwent patch tests with all 12 chemicals at the beginning of the study. Three individuals displayed a “manifest” allergy to any one of the chemicals, whereas 9 exhibited a “latent” allergy (the terms “manifest” and “latent” not being explained). Testing was repeated when the subjects completed their studies. At that point, 7 of the participants showed a “manifest” allergy. A “latent” allergy was observed in 43 of the participants. The percentage of female participants with positive reactions was very high in relation to the percentage found in males. Trioxane, which was associated with a rate of 28.8%, had the greatest allergic potential, followed by chlorophenol-campher (25.8%), eugenol (22.7%) and amalgam (15.2%). The results were not broken down into “manifest” and “latent” allergies and no indication was given as to what served as the basis for the percentages (no further details; Dzhemileva et al., 1976).

9 Classifications and threshold limit values

Trioxane has been legally classified in the TRGS 905 and placed into category R_D3 of substances toxic to reproduction (i.e. “substances which cause concern for humans owing to possible developmental toxic effects”) in accordance with the EU classification criteria (TRGS 905, 2002).

In Poland, based on the subchronic inhalation studies in rats discussed in Section 7.5, levels of 15 mg/m³ (time-weighted average) and 75 mg/m³ (short-term exposure limit) were suggested (Indulski et al., 1986) and later established (RTECS, 2002) as the maximum allowable concentrations of trioxane.

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