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TOXICOLOGICAL EVALUATIONS

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TOXICOLOGICAL EVALUATION

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Chloroacetamide No. 8

CAS No. 79-07-2

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Chloroacetamide

1 Summary and assessment

Chloroacetamide proves to be toxic upon acute oral administration and dermal application. The oral LD_{50} values for the rat and the mouse are 138 mg/kg body weight and 150 mg/kg body weight, respectively. Following application of 400 mg chloroacetamide/kg body weight to the skin of rabbits, 3 out of 5 animals died after 1 to 3 treatments.

Chloroacetamide has a slightly irritant effect on the skin when made into a paste with physiological saline solution and applied to the shorn skin of rabbits. Studies with diluted solutions of chloroacetamide indicate a skin-irritating potential in rabbits and guinea pigs following a single application. On repeated application to the skin of the rabbit, inflammatory degenerative skin changes develop. Chloroacetamide has an irritant effect on the eye. In the Magnusson and Kligman maximisation test carried out in accordance with OECD guideline No. 406, chloroacetamide causes clear sensitisation of the guinea pig skin. This is in contrast, however, to less recent studies which were not carried out in accordance with current guidelines for testing. Those studies did not show the substance to have any skin-sensitising potential. Chloroacetamide is therefore now assessed as having a skin sensitising potential in animal studies.

In 30-day studies in female rabbits, lethal doses of ≥ 25 mg/kg body weight resulted in dose-dependent paralysis of the extremities following subcutaneous and intravenous administration of chloroacetamide. However, no histopathological changes were observed in the organs, and urinalysis findings were normal. Upon treatment with 25 mg/kg body weight, haemoglobin content was decreased. All animals treated with higher doses (50 and 100 mg/kg body weight) died. In a corresponding study with dermal application, fatty infiltration of the liver and the heart and deposition of haemosiderin in the spleen were observed at doses of 100 mg/kg body weight and above. Doses of up to 10 mg/kg body weight/day (subcutaneous and intravenous administration) were tolerated by rabbits without causing any damage; 25 mg/kg body weight/day applied to the skin caused local but not systemic changes. In a 90-day feeding trial in rats, a daily dose of approx. 50 mg/kg body weight resulted in retarded body weight development, leu-

kocytosis and, in the male rats, testicular damage. These signs of toxicity were not fully reversible even after a follow-up observation period of 4 weeks. Daily doses of 10 mg/kg and 20 mg/kg body weight were tolerated without any substance-related changes. These findings were confirmed by a further study in rats which was conducted over a period of 3 months. In that study, a daily dose of 50 mg chloroacetamide/kg body weight caused a marked reduction in testicle weights in the males as well as severe impairment of spermatogenesis. The female rats reacted to the substance with enlarged thyroid glands. These effects were seen even at the low dose of 12.5 mg/kg body weight, but they were less pronounced. No other types of substance-related damage have been detected.

In the Salmonella/microsome assay in vitro, chloroacetamide does not exhibit any genotoxic potential, either with or without metabolic activation. In vivo, as well, chloroacetamide is not mutagenic in the Chinese hamster chromosome aberration test with examination of the bone marrow and the spermatogonia, in the Chinese hamster and mouse micronucleus tests and in the dominant lethal test in the mouse. Only one in vitro fluctuation test in *Klebsiella pneumoniae* has given a positive result. Based on the available data, chloroacetamide can not be assumed to have a mutagenic potential.

Following single subcutaneous or intraperitoneal administration on particular days of gestation in exploratory teratogenicity studies, one of which included subsequent rearing, no indications of a teratogenic potential were found. However, 50% of the pups in the litters died after birth if the mothers were treated with a high dose of chloroacetamide (approx. 70% of the LD_{50}). In the dominant lethal test, if male mice are treated once with an intraperitoneal dose of chloroacetamide in the LD_{50} range and then mated at intervals of one week, decreases in fertility index and in the numbers of implantations and viable foetuses are observed in the first three weeks. Treatment of male rats with otherwise hardly toxic doses of chloroacetamide (2, 10 and 50 mg/kg body weight/day) for 3 months was observed to result in a reduction in testicular and epididymal size and weight at the highest dose. Histopathological examination revealed severe impairment of spermatogenesis, and cytotoxicity. At the end of the 29-day observation period there were signs of partial regeneration of the testicular tubules. In a further study, in which male rats were similarly treated for 3 months with 12.5 and 50 mg/kg body weight per day, both dosages resulted in dose-dependent reduction in testicular weights and severe impairment of spermato-

genesis to the extent of complete absence of fully mature sperm cells. The results demonstrate that chloroacetamide causes considerable damage to the testes of the rat and is capable of producing male hypofertility in experimental animals.

High doses of chloroacetamide lead to reduction in glutathione content in hepatocytes, increased lipid peroxidation and degenerative changes in the liver, both in vitro and in vivo.

In humans, diluted aqueous solutions of chloroacetamide are not irritating to the skin. However, numerous reports demonstrate that chloroacetamide can produce contact allergies in humans.

In the Federal Republic of Germany, chloroacetamide has been legally classified in the TRGS 905 and placed into category R_F3 of substances toxic to reproduction (i.e. “substances which cause concern for human fertility”) in accordance with the EU classification criteria; furthermore it has been assigned the risk phrase R43 indicating sensitisation.

2 Name of substance

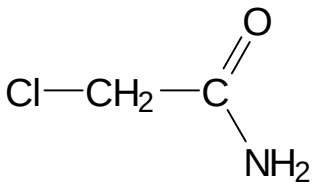
2.1	Usual name	Chloroacetamide
2.2	IUPAC name	2-Chloroacetamide
2.3	CAS No.	79-07-2
2.4	EINECS No.	201-174-2

3 Synonyms, common and trade names

Chloressigsäureamid
Chloracetamid
2-Chloracetamid
α-Chloroacetamide
Mergal AF
Microcide

4 Structural and molecular formulae

4.1 Structural formula



4.2 Molecular formula C_2H_4ClNO

5 Physical and chemical properties

5.1	Molecular mass, g/mol	93.5	
5.2	Melting point, °C	121 114–118	(Lide and Frederikse, 1996) (Hoechst, 1981)
5.3	Boiling point, °C	225 Decomposition temperature above melting temperature	(Lide and Frederikse, 1996) (Hoechst, 1981)
5.4	Vapour pressure, hPa	0.07 (at 20 °C)	(Hoechst, 1981)
5.5	Density, g/cm³	No information available	
5.6	Solubility in water	Dissolves well 90 g/l (at 20 °C) 170 g/l (at 40 °C)	 (Hoechst, 1981)
5.7	Solubility in organic solvents	Dissolves well in dimethyl sulfoxide and N-dimethyl formamide, in isopropanol 40 g/l (at 20 °C), in butanol 35 g/l (at 20 °C)	(Hoechst, 1977)
5.8	Solubility in fat	No information available	
5.9	pH value	3.8–4.1	(Hoechst, 1985)
5.10	Conversion factor	–	

6 Uses

Very widely used as a preservative, e. g. in cosmetics, pharmaceuticals, adhesives, household cleaning agents and industrial oils (Hoechst, 1977; Berndt, 1991; BgVV, 1999).

7 Experimental results

7.1 Toxicokinetics and metabolism

No information available.

7.2 Acute and subacute toxicity

Acute toxicity

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

Table 1. Acute toxicity of chloroacetamide

Species, strain, sex*	Route	Dose (mg/kg b. w.)	Effect	Observation period	Reference
Rat, Wistar, female	oral	138	LD_{50}, no further data	14 days	Hoechst, 1976
Mouse	oral	150	LD_{50}, no further data	no details	Hoechst, 1981
Rat, Long Evans	intraperitoneal	50	LD_{50}, no further data	no details	Thiersch, 1971
Mouse	intraperitoneal	100	LD_{50}, no further data	no details	RTECS, 1997
Mouse	intravenous	180	LD_{50}, no further data	no details	RTECS, 1997
Rat	subcutaneous	70	LD_{50}, no further data	no details	von Kreybig et al., 1969
Mouse	no details	155	LD_{50}, no further data	no details	Chung-hua, 1978
Rabbit	no details	122	LD_{50}, no further data	no details	Chung-hua, 1978
Dog	no details	31	LD_{50}, no further data	no details	Chung-hua, 1978

* where specified
b. w. body weight

Data on signs of toxicity, necropsy findings and observation period are lacking. In part, the references do not state the route of administration. Nonetheless, it may be concluded from the quite consistent findings that chloroacetamide is a substance which causes acute toxicity and hence is to be considered “toxic”. Determination of the inhalation toxicity of chloroacetamide is not possible because the chemical has a very low vapour pressure.

Subacute toxicity

Groups of 5 female rabbits (Yellow-Silver, weighing between 1.6 and 2.9 kg) received dermal applications of 25, 50, 100, 200 and 400 mg chloroacetamide/kg body weight to the depilated skin of the neck on 30 consecutive days. In order to prevent the animals from licking off the chemical, they had plastic cuffs fitted around their necks. The various doses of chloroacetamide were applied as 40-percent suspensions in water. Body weights were determined three times a week. Before commencement of the study and after the end of dosing, blood tests (haemoglobin, red blood cells, white blood cells, differential blood count, Heinz bodies) and urine tests (protein, glucose, sediments) were carried out. The heart, lung, liver, spleen, kidney, adrenal glands, ovaries, pancreas, brain, pituitary gland and thymus gland of each animal were histopathologically examined. The findings are shown in Table 2.

Table 2. Findings following 30-day dermal application of chloroacetamide in rabbits (Hoechst, 1967 a)

Dose (mg/kg body weight)	Mortality	Findings
25	0/5	encrustation and thickening of the application site
50	1/5	encrustation and thickening of the application site, but no other findings
100	0/5	encrustation and thickening of the application site, body weight reduction; fatty infiltration of the liver and heart, deposition of haemosiderin in the spleen
200	0/5	same changes as seen after 100 mg/kg body weight
400	3/5 (after 1 to 3 treatments)	encrustation and thickening of the application site, body weight reduction, fatty infiltration of the liver and heart, deposition of haemosiderin in the spleen
Controls (untreated)	0/5	no findings

Thirty dermal applications of chloroacetamide at dose levels of 25 and 50 mg/kg body weight (as 40-percent aqueous suspensions) were tolerated without absorption-related injury, except for local skin changes. At and above the 100 mg/kg body weight level, dose-dependent body weight loss was observed, as were histopathological changes of the liver, heart and spleen. The 400 mg/kg body weight dose was lethal in 3 out of 5 animals. The local changes as well as the liver, kidney and spleen histopathology findings were more marked than those seen at the lower doses. Analysis of blood and

urine revealed no pathological findings in any of the dose groups (Hoechst, 1967 a).

In a study in female rabbits, chloroacetamide was administered subcutaneously for 30 consecutive days at dose levels of 2, 10, 25, 50 and 100 mg/kg body weight (controls receiving 1 ml 0.9-percent saline/kg body weight; experimental setup, study parameters, histology, etc., as in the 30-day dermal application study; Hoechst, 1967 a). The findings are shown in Table 3 below.

Table 3. Findings following 30-day subcutaneous administration of chloroacetamide in rabbits (Hoechst, 1967 b)

Dose (mg/kg body weight)	Mortality	Findings
2	0/5	no findings
10	0/5	no findings
25	1/5	hindlimb paralysis, diarrhoea, thickening at the injection site; reduced haemoglobin levels
50	5/5 (after 7 to 13 injections)	forelimb and hindlimb paralysis, skin of the neck thickened, diarrhoea; no histopathological changes; no laboratory tests carried out
100	5/5 (after 1 or 2 injections)	no signs of toxicity reported; no laboratory tests or post-mortem conducted
Controls (physiological saline)	0/5	no findings

Subcutaneous administration of chloroacetamide thus resulted in dose-dependent signs of paralysis and death of the experimental animals at levels of 25 mg/kg body weight and above. A harmful effect on the blood count can not be excluded. Histopathological examination of the organs did not reveal any changes. Urinalysis was without findings (Hoechst, 1967 b).

In a corresponding 30-day study (with the same experimental setup, study parameters, histology, etc. as in the subcutaneous administration study; Hoechst, 1967 b), chloroacetamide was administered to female rabbits by intravenous injection on 30 consecutive days. The findings are shown in Table 4.

Table 4. Findings following 30-day intravenous administration of chloroacetamide in rabbits (Hoechst, 1967 c)		
Dose (mg/kg body weight)	Mortality	Findings
2	0/5	no findings
10	0/5	no findings
25	1/5	limb paralysis starting after injection 13, diarrhoea, body weight loss; slight reduction in haemoglobin; organs were without histopathological findings
50	5/5 (after 6 to 15 injections)	limb paralysis starting after injection 7, diarrhoea; no laboratory tests were carried out; organs were without histopathological findings
100	5/5 (after 1 or 2 injections)	no signs of toxicity reported; no laboratory tests or post-mortem conducted
Controls	0/5	no findings

Thirty intravenous administrations of chloroacetamide to rabbits resulted in dose-dependent signs of paralysis and death of the experimental animals at levels of 25 mg/kg body weight and above. Histopathological examination of the organs did not reveal any changes. Urinalysis was without findings (Hoechst, 1967 c).

7.3 Skin and mucous membrane effects

A primary skin irritancy study of chloroacetamide was conducted in 3 New Zealand white rabbits (2.5 to 2.8 kg, aged 3 to 5 months) in accordance with OECD guideline No. 404. Each animal had 500 mg chloroacetamide (made into a paste with 0.25 ml 0.9-percent saline solution) applied to the shorn skin in the dorsal region of the trunk, the treated area being covered with a semi-occlusive dressing. After an exposure period of 4 hours, the dressing was removed and the skin washed off with lukewarm water. The findings were assessed 30 minutes, 1 hour and 1, 2, 3 and 7 days after removal of the dressing. One of the rabbits showed very mild erythema and oedema formation 1 hour to 3 days after removal of the dressing, the second exhibited moderate erythema and oedema formation while the third animal displayed severe erythema and moderate oedema formation. All signs of irritation were reversible 7 days after removal of the dressing. Accordingly, the authors evaluated chloroacetamide as slightly irritating (Hoechst, 1993 a).

In a subacute toxicity study (cf. Section 7.2), female Yellow-Silver rabbits (1.9 to 3.1 kg) received daily applications of 40-percent aqueous chloroacetamide suspensions to the depilated skin of the neck, plastic cuffs then being placed around the animals' necks in order to prevent them from licking off the chemical. In the lowest dose group (0.063 ml suspension/kg body weight, equivalent to 25 mg/kg body weight), "encrustations" of the skin were observed as early as the first day. As of treatment days 4 or 5, parts of the treated skin areas were "hardened" (no further details; Hoechst, 1967 a).

When guinea pigs (Pirbright White) received single applications of aqueous chloroacetamide solution to the dorsal skin for 24 hours, erythema was observed following exposure to 0.5 ml of a 25-percent solution. A 9-percent solution did not produce any effects (IBR, 1985).

A maximisation test of chloroacetamide in the guinea pig employed acetone as the solvent. It was reported that a 3-percent solution induced an inflammatory skin reaction in the controls (no further details; Agren et al., 1980).

"CA 24" (a mixture of 70% chloroacetamide and 30% sodium benzoate), applied as a 5-percent aqueous solution (equivalent to 3.5% chloroacetamide and 1.5% sodium benzoate), caused no skin irritation in the Draize test (24-hour occlusive application to the shaved and scarified skin of the rabbit; IBR, 1981 a).

An eye irritation study of chloroacetamide was conducted in 3 New Zealand white rabbits (weighing between 2.9 and 3.4 kg, aged 3 to 5 months) in accordance with OECD guideline No. 405. Each animal had a single dose of 100 mg chloroacetamide instilled into the conjunctival sac of its left eye. After 24 hours, the eye was rinsed out thoroughly with physiological saline solution. The damage to the eye was assessed 1, 24, 48 and 72 hours following application. The right eye served as the control. After 1 to 7 hours the animals displayed severe reddening and swelling of the conjunctiva. In addition, corneal clouding was seen. The effects cleared up only slowly but were reversible after 21 days following instillation. Accordingly, the authors evaluated chloroacetamide as irritating to the eye (Hoechst, 1993 b).

When applied to the mucous membranes of the rabbit eye (6 albino rabbits), 0.1 ml of a 5-percent "CA 24" solution (composition as described above) caused no irritation (IBR, 1981 b).

7.4 Sensitisation

The potential of chloroacetamide to cause skin sensitisation was studied in groups of 5 guinea pigs (Dunkin-Hartley strain, 250 to 450 g) receiving intradermal treatment with Freund's adjuvant and aqueous solutions of chloroacetamide (analytical grade) in the Magnusson and Kligman maximisation test (OECD guideline No. 406). Chloroacetamide concentrations of 0.003, 0.01, 0.03, 0.1 and 0.3% were used. The control group was treated with Freund's adjuvant only. Seven days later, chloroacetamide was applied without adjuvant to the skin of the experimental animals as a 0.3 or 30% solution in a 1 : 7 polyethylene glycol/water mixture and left for 48 hours under occlusive cover. The controls received the vehicle only. After another 12 days, the sensitisation reaction was induced in all groups, including the controls, with the aid of a 5-percent chloroacetamide solution in a 1 : 7 polyethylene glycol/water mixture by exposing the shorn skin to the solution for 24 hours under occlusive cover. The results were assessed 24, 48 and 72 hours after removing the dressing. Rechallenge was carried out in the same way 7 days after the first challenge. Irrespective of the chloroacetamide concentrations used, 60 to 100% of the chloroacetamide-treated animals showed a positive response after the initial challenge (controls: 0/5). At rechallenge, the initial challenge proved to have induced sensitisation in the controls, with 80% of the animals showing a positive reaction. From these results, the authors concluded that chloroacetamide has a strong sensitising potential, as a high percentage of the animals were sensitised in this study and even the controls developed an allergy to chloroacetamide subsequent to the first challenge dose and showed clearly positive responses after rechallenge (Boman et al., 1996).

The following four studies with largely negative results do not satisfy the requirements of the testing guidelines which have become common standard in recent years, and they have considerable methodological flaws. For instance, the concentrations of chloroacetamide employed to induce and trigger the allergic reaction, generally, were too low. Hence, it cannot be concluded that chloroacetamide can not be demonstrated to have a sensitising potential in animals.

In a skin sensitisation study according to the method developed by Magnusson and Kligman, chloroacetamide was tested in 20 guinea pigs (Pirbright White) and appropriate controls. Induction treatment was carried

out with a 9-percent aqueous solution, which in the preliminary primary irritancy study had been tolerated without eliciting a response, whereas the 25-percent and 50-percent solutions and the undiluted product had caused erythema (following 24-hour exposure). Testing for positive skin sensitisation was carried out by means of the closed patch test 14 days after the last exposure, using chloroacetamide concentrations of 3, 1 and 0.3% in water (in accordance with the sponsor's instructions). No signs of skin sensitisation were observed (IBR, 1985).

In an open epicutaneous test without Freund's adjuvant, groups of 10 female albino guinea pigs (Pirbright White) received local exposures of the shaved skin of the flank to 3% or 1% chloroacetamide solutions (no details of product purity or skin reactions given) 5 times per week over a period of 4 weeks. Ten days after completion of the pretreatment, a 0.2-percent solution was applied to the contralateral, untreated skin of the flank in order to elicit the positive response. No definite allergic reaction was elicited in either group of pre-treated guinea pigs when a 0.2-percent solution was used. In a very short expert's report the author remarks in his discussion that "it has previously been established that if Freund's adjuvant is used, a sensitising effect of chloroacetamide can be demonstrated in guinea pigs" (no further details; Schulz, 1984).

There is a further study on the sensitising potential of the preservative, "CA 24" (70% chloroacetamide and 30% sodium benzoate), which was conducted according to a modification of the Buehler method. Twenty guinea pigs were treated dermally with 0.5 ml 0.3-percent "CA 24" solution (concentration indicated by the sponsor), applied to the shorn skin of the left flank once a week over a period of three weeks. The treated skin area was kept covered for 6 hours. Fourteen days after the last treatment, the 20 treated guinea pigs and 10 controls received corresponding applications of 0.3-percent "CA 24" solution to the right flank, and 2, 24 and 48 hours later the skin reactions were compared with those of the controls. No signs of skin sensitisation were observed (no further details; IBR, 1981 c).

Guinea pigs were also exposed to chloroacetamide in a modified Draize test. Ten animals of approximately 350 g underwent exposure to chloroacetamide in two cycles of induction and challenge (no details of the concentrations used). One of the 10 animals showed a positive allergic reaction. The authors themselves discussed their method as not being very

sensitive and conceded that the negative result must be interpreted with caution (Johnson and Goodwin, 1985).

7.5 Subchronic and chronic toxicity

In a 90-day feeding trial in rats (young Wistar rats, Hoe: WISKf(SPF71) strain, approximately 80 g, 10 males and 10 females per dose group), chloroacetamide doses of 0 (controls), 20, 100 and 500 mg/kg feed (equivalent to 0, 2, 10 and 50 mg/kg body weight/day, according to the authors) were administered for 90 days. At the end of the treatment period, 5 male and 5 female rats were placed under observation for 29 days. An adverse effect on body weight gain was only seen in the males and females of the top dose group (500 mg/kg feed = 500 ppm). Feed consumption was reduced during the first 30 days at this dose level. From the haematological and clinico-chemical tests and the urinalyses conducted at the end of the study there were no indications of substance-related changes, with the exception of leukocytosis in the male and the female rats, but this finding was reversible. At the end of the feeding study, the male rats of the highest dose group were observed to have a significant reduction in testicular weight which was still present after a 29-day observation period. A reduction in testicular and epididymal size was seen. Histopathological examination revealed depression and/or cessation of spermiogenesis as well as moderate proliferation of Leydig's cells immediately after the end of the study. The epididymis contained neither mature nor immature sperm cells, only cross-linked protein. At the end of the 29-day observation period there were signs of partial regeneration of the testicular tubules. No other histopathological changes were observed in the males. The females of all dose groups were without histopathological findings at both time points of examination. Follicle maturation was found to be normal. In the groups receiving 100 and 20 mg/kg feed (approximately 10 and 2 mg/kg body weight per day), no substance-related organ changes were seen (Hoechst, 1985).

A short summary report describes a study in Sprague-Dawley rats which received chloroacetamide in the feed over a period of 3 months. Groups of 10 female and 10 male rats were treated with daily doses of 0 (controls), 12.5 and 50 mg chloroacetamide/kg body weight. In the group receiving 50 mg/kg body weight, mild sedation was seen as of treatment week 4 to 6, body weight gain was significantly retarded and feed consumption was re-

duced. At terminal necropsy, the males were found to have markedly reduced testicular weights while the females showed enlarged thyroid glands. The animals having received treatment with the lower dose of 12.5 mg/kg body weight displayed the same effects in a clearly discernible but less pronounced form. Behavioural observation, haematology and clinico-chemical tests, urinalysis as well as hearing and visual tests gave no indications of effects related to the test substance. Histopathological examination revealed that spermatogenesis was severely impaired in a dose-dependent manner to the extent of complete absence of mature sperm cells. The other organs showed no histopathological changes (Hoechst, 1992).

In a 90-day dermal application study, the commercially available preservative, “CA 24” (a mixture of 70% chloroacetamide and 30% sodium benzoate, no details given regarding the purity of the two components), was applied to the shorn skin of male Wistar rats weighing approximately 210 g (no details of the number of applications per week). Groups of 10 rats were treated with 3% and 1% solutions of “CA 24” in Lanette and a 2% solution in distilled water (equivalent to chloroacetamide concentrations of 1.4% and 0.7% in Lanette and 1.4% in distilled water). A control group was treated with Lanette. “CA 24” was applied at dose levels of 50 mg/kg body weight (35 mg chloroacetamide/kg body weight) and 12.5 mg/kg body weight (8.75 mg chloroacetamide/kg body weight) in Lanette and 50 mg/kg body weight (35 mg chloroacetamide/kg body weight) in aqueous solution. No reasons were given for the choice of dosages. The study parameters, which included body weight, feed consumption and histopathological examination of the testis and epididymis, gave no indications of pathological changes attributable to the substance. There are no data on the chemical’s dermatological actions during the treatment (no further details; IBR, 1971).

7.6 Genotoxicity

7.6.1 In vitro

In the Ames Salmonella/microsome assay (standard plate incorporation test) chloroacetamide concentrations in the range from 4 to 2500 µg/plate showed no mutagenic potential in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537 with or without metabolic activation (S-9 mix from Aroclor 1254-induced rat liver; Hoechst, 1979). No data were reported

to show whether any of the chloroacetamide concentrations were within the toxic range.

In the Ames Salmonella/microsome assay, which was conducted as a standard plate incorporation test, the preservative “CA 24” (a mixture of 70% chloroacetamide and 30% sodium benzoate) proved to be devoid of mutagenic potential at concentration levels of 0 to 1000 µg/plate (0.35 to 700 µg chloroacetamide/plate) both in the presence and absence of S-9 mix, the higher dose levels exhibiting bacteriotoxic effects (no further details; IBR, 1979 a).

In a further standard plate incorporation test using *Salmonella typhimurium* strains TA 98, TA 100 and TA 1537 with and without metabolic activation with S-9 mix from Aroclor-induced rat liver, chloroacetamide caused no detectable mutagenic effect. The tests were carried out with chloroacetamide concentrations ranging from 0.05 to 10 mg/plate. A concentration of 20 mg/plate stopped bacterial growth. There was no case in which chloroacetamide was detected to have led to increased revertant counts (Voogd et al., 1989).

In a fluctuation test using *Klebsiella pneumoniae* chloroacetamide was detected to possess mutagenic activity. The bacteria were incubated for 20 hours in beef bouillon to which chloroacetamide concentrations of 0.1 to 2 g/l had been added. Subsequent incubation on agar plates which had partly been prepared with streptomycin was used to determine the number of bacteria to have acquired streptomycin resistance by mutation. In the presence of chloroacetamide concentrations of and above 0.5 g/l the mutation rate increased up to tenfold in a concentration-dependent manner. At that concentration, chloroacetamide did not exhibit any marked bacteriotoxicity. At 2 g/l, however, 80 to 85% of the bacteria were destroyed (Voogd et al., 1989).

7.6.2 In vivo

According to a report which was available only as a summary, 2 intraperitoneal injections of the preservative, “CA 24” (70% chloroacetamide, 30% sodium benzoate), administered at an interval of 24 hours at dose levels of 50 mg/kg body weight (1/3 of the LD50, equivalent to 35 mg chloroacetamide/kg body weight), 25 mg/kg body weight (1/6 of the LD_{50}, equivalent to 17.5 mg chloroacetamide/kg body weight) and 12.5 mg/kg body weight (1/12 of the LD_{50}, equivalent to 8.75 mg chloroacetamide/kg body weight) caused no

structural or numerical chromosome aberrations in the bone marrow and spermatogonia of Chinese hamsters, and in addition no increase was observed in the number of micronuclei in the bone marrow (no details of experimental conduct, data evaluation, etc. were provided; IBR, 1979 b).

A further micronucleus test with chloroacetamide (technical grade) was conducted in NMRI mice. Groups of 5 male and 5 female mice were dosed twice at an interval of 24 hours with doses of 0 (controls), 1, 10 or 100 mg chloroacetamide/kg body weight by oral gavage. Preliminary range-finding studies had shown two administrations of 100 mg/kg body weight to be the maximum tolerated dose. Positive controls were treated with cyclophosphamide at a dose level of 100 mg/kg body weight. Six hours following the second administration, the animals were sacrificed, and smears of the femural bone marrow were prepared on slides and stained for microscopy. Per animal, 2000 polychromatic erythrocytes were examined for micronuclei, and the ratios of juvenile forms and normocytes were determined, as were the numbers of normocytes containing micronuclei. Chloroacetamide exposure did not lead to significant changes in any of the measured parameters compared with controls, whereas cyclophosphamide caused a marked increase in the number of micronuclei (Hoechst, 1982).

In a dominant lethal test with the preservative, “CA 24” (70% chloroacetamide, 30% sodium benzoate), groups of 10 male NMRI mice (weighing approx. 20 g) were given single intraperitoneal injections of 114 and 123 mg/kg body weight (equivalent to 79.8 and 86 mg chloroacetamide/kg body weight, respectively). The controls received 10 ml/kg body weight of the 0.5% methylcellulose used in preparing the suspension. The LD_{50} (24 hours) was determined as 130 mg/kg body weight (91 mg chloroacetamide/kg body weight). The treated male mice were each mated with 3 virgin females per week over a period of 10 weeks. Implantations were reduced at the end of weeks 1, 2 and 3, and a reduction in fertility index was seen after weeks 1 and 2. In addition, the number of foetuses after weeks 1, 2 and 3 were clearly lower compared with the controls. After week 4, the effects were no longer observed. In the authors’ opinion, these changes were attributable to a toxic effect of “CA 24” on the males during the first 3 weeks. The number of resorptions, the mutagenicity index and the number of dead developed foetuses remained unchanged throughout the entire study, which led the authors to conclude that a mutagenic effect seemed unlikely (no further details; IBR, 1979 c).

7.7 Carcinogenicity

No information available.

7.8 Reproductive toxicity

In order to test chloroacetamide for teratogenicity, rats (CD and BDIX strains, numbers not given) were treated with a single subcutaneous dose of up to 50 mg/kg body weight, equivalent to approximately 71% of the LD_{50} (LD_{50} subcutaneous 70 mg/kg body weight) on days 13 or 14 of gestation. The dose proved toxic and resulted in postnatal death in 50% of the pups. There were no cases of malformations. Development was normal in the survivors. No details were given either of the criteria used to assess the pups which died postnatally and those which survived, or of the criteria employed to evaluate toxicity in the dams (von Kreybig et al., 1968, 1969).

When chloroacetamide (LD_{50} intraperitoneal 50 mg/kg body weight) was administered intraperitoneally to pregnant Long-Evans rats at 20 mg/kg body weight either as a single dose on day 7 or on days 11 and 12 of gestation and caesarean section was performed in both groups on day 21 of gestation, assessment of implantations, placental weights, foetal weights and foetal malformations revealed no cases of embryotoxicity or teratogenicity. Moreover, the dams showed no signs of toxicity (no further details; Thiersch, 1971).

In two available 90-day studies (see also Section 7.5) in which rats were treated with chloroacetamide doses of up to 50 mg/kg body weight in the feed, marked reductions in testicular weights were observed at the end of the study. A reduction in testicular and epididymal size was seen. Histopathological examination revealed very severe impairment of spermatogenesis and, in general, marked testicular cytotoxicity. In one of the two studies, the effects did not occur at the low 2 and 10 mg/kg body weight doses, and at the high 50 mg/kg body weight dose signs of partial regeneration of the testicular tubules were seen at the end of a 29-day observation period. In the females, follicle maturation was found to be normal (Hoechst, 1985). In the second of the two studies, the testicular damage observed at 50 mg/kg body weight was also seen following administration of 12.5 mg/kg body weight, though they were less pronounced at the latter dose (Hoechst, 1992).

In a dominant-lethal test (see also Section 7.6.2), in which male mice received single intraperitoneal administrations of chloroacetamide (123 mg "CA 24"/kg body weight) and were subsequently mated with untreated females for several weeks, there were reductions in fertility index as well as in the numbers of implantations and viable foetuses in the first 2 to 3 weeks following administration. When mating took place 4 weeks after the males had been treated, no effects were observed. In the authors' interpretation, these findings reflected the toxic impairment of the males during the first 3 weeks after chloroacetamide treatment (IBR, 1979 c).

7.9 Effects on the immune system

No information available.

7.10 Neurotoxicity

No information available.

7.11 Other effects

Chloroacetamide leads to a reduction in glutathione content and enhancement of lipid peroxidation in hepatocytes, and consequently causes damage to the cells, both in vitro and in vivo.

Primary rat hepatocytes, obtained from Sprague-Dawley rats by means of the collagenase perfusion method, were incubated for 4 to 5 hours with serum to which 18.7 µg chloroacetamide/ml had been added. There was a very sharp drop in cellular glutathione content in the first hour of incubation, a considerable enhancement of lipid peroxidation (measured as the accumulation of malondialdehyde in the incubated cells) and marked lysis of the treated cells. By adding methionine, which stimulates hepatocellular glutathione synthesis, it was possible to achieve clear inhibition of all of the effects described (Anundi et al., 1979).

Following single intraperitoneal administration of 75 mg chloroacetamide/kg body weight to male Sprague-Dawley rats (180 to 200 g), hepatic glutathione content fell by approximately 90% in the first hour, but returned to slightly above normal values within 48 to 72 hours. Three to six hours after

chloroacetamide administration, lesions developed in the peripheral midzonal parts of the hepatic lobules (swelling and hydropic degeneration). During this period, an enhancement of lipid peroxidation was measured by means of the thiobarbituric acid test. Hydropic degeneration of the hepatic lobules showed remission by 1/3 one week after treatment. The rats' response was augmented after a 24-hour fasting period. A single intraperitoneal dose of 37.5 mg chloroacetamide/kg body weight had no effect on rat liver cell morphology. Following intraperitoneal administration of 112.5 mg/kg body weight mortality rose to high levels within 5 to 6 hours, and histopathological examination of the livers of these animals revealed fatty degeneration, necrosis and leukocytic infiltration. Repeated intraperitoneal injections of 37.5 mg chloroacetamide/kg body weight every second day also resulted in hepatocellular swelling and hydropic degeneration within 2 weeks. These changes were comparable with those seen after a single dose of 75 mg/kg body weight (Anundi et al., 1980).

Another study in male Sprague-Dawley rats (210 to 260 g) investigated the effect of chloroacetamide on lipid peroxidation in vivo by means of the ethane/pentane breath test. Chloroacetamide was administered intraperitoneally at dose levels of 70, 80 and 150 mg/kg body weight. Upon treatment, the animals were immediately placed in closed breathing chambers with circulating air, and the amounts of ethane and pentane exhaled over periods of 2 and 4 hours were measured. Increases in the exhaled amounts of ethane and pentane were only small at 4 hours after the 70 mg/kg body weight dose, marked at 4 hours after the 80 mg/kg body weight dose and very marked at only 2 hours after the 150 mg/kg body weight dose. At the highest dose level, the animals died after 2 hours. The authors therefore saw the findings of Anundi et al. (1980) regarding chloroacetamide-induced stimulation of lipid peroxidation in vivo confirmed by an independent method (Cluet and Boudène, 1983).

8 Experience in humans

In a study conducted to investigate the skin-damaging potential of the preservative, "CA 24" (70% chloroacetamide and 30% sodium benzoate), 10 subjects without dermatological problems were treated daily for 14 days with a 0.1-percent aqueous solution of "CA 24" which was applied to the

same skin area and then rubbed into the skin. None of the subjects developed any signs of irritation at this very low concentration (Röckl, 1970).

The potential of chloroacetamide to cause contact allergies in humans is addressed in a large number of case reports and reports of positive patch tests in different groups of patients and healthy volunteers. Table 5 below lists examples of case reports from the literature published in the period from 1971 to 1999. No claim is made that the compilation is exhaustive.

Beginning of Table 5

Table 5. Chloroacetamide-induced allergic skin reactions – case reports –

Patient (age)	Observed lesion	Contact with chloroacetamide	Concentration eliciting a positive patch test response	Reference
Female (25)	dermatitis on the sides of the fingers	body lotion for babies	0.18%	Nater, 1971
Male (45)	hand dermatitis	wallpaper glue	0.10%	Bang Pedersen and Fregert, 1976
Male (52)	hand dermatitis	wallpaper glue	0.10%	Bang Pedersen and Fregert, 1976
Female (27)	eczema of the fingertips	body massage cream	0.10%	Dooms-Goossens et al., 1981
Male (30)	dermatitis of the backs of the hands	cosmetic hand lotion	0.20%	Suhonen, 1983
Female (40)	facial dermatitis	facial aerosol spray astringent	0.20%	Koch et al., 1985
Male (34)	hand dermatitis	commercial cutting oil	2.00%	Lama et al., 1986
Female (46)	burning swelling of the eyelids	anti-wrinkle cosmetic	0.20%	de Groot and Weyland, 1986
Female (25)	erythema in the perioral area, on both ears	ointment for treatment of herpes	0.07%	Detmar and Agathos, 1988
Male (48)	eczema of the hands	paint	0.20%	Jones and Kennedy, 1988
Male (27)	dermatitis of the dorsa and the soles of the feet	impregnated shoe leather (suspected)	0.20%	Jelen et al., 1989
Male (71)	redness, swelling and scaling of the border of the lips	toothpaste	0.20%	Machácková and Smid, 1991
Male (51)	dermatitis of the forearm	gel for mosquito bites	0.001%	Wantke et al., 1993
Female (33)	dermatitis of the face and neck	ecofriendly paint ("Umwelt-Raumfarbe")	0.10%	Finkbeiner and Kleinhans, 1994
Female (55)	dermatitis of the face, hands and armpits	roll-on deodorant	0.20%	Taran and Delaney, 1997

Table 5. Chloroacetamide-induced allergic skin reactions – case reports –				
Patient (age)	Observed lesion	Contact with chloroacetamide	Concentration eliciting a positive patch test response	Reference
Female (55)	dermatitis in the neck region	hair colorant	no information	Assier-Bonnet and Revuz, 1999
Male (36)	dermatitis of the palms and fingertips	wallpaper glue	0.20%	Pereira et al., 1999

End of Table 5

A further example is that of a Dutch report. In the Netherlands, a total of 27 patients examined in the period from August, 1969, to December, 1970, had developed a contact allergy to hirudin-containing ointments used in the treatment of thrombophlebitis and haematoma. In 17 of the patients, it was possible to demonstrate clearly that the allergy was attributable to the chloroacetamide contained in the ointments as a preservative (Smeenk and Prins, 1972).

Table 6 summarises findings in patients who were tested to establish whether or not they had developed hypersensitivity to chloroacetamide. Again, this compilation is not exhaustive and must be regarded as a collection of examples. The table shows that in the patient groups treated at dermatology departments, individuals with acquired contact allergies to chloroacetamide are regularly seen. The frequency of occurrence in the patient groups varies within the range from 0.3 to 2.8%.

Beginning of Table 6

Table 6. Frequency of chloroacetamide-induced contact allergy in different patient groups					
Number of individuals tested	Type of group	Pre-existing lesion	Test concentration	Number of positive tests	Reference
209	patients	eczema	0.35%	none	Schmidt-La Baume and Pfister, 1966
200	inpatients	various skin diseases	0.07%	none	Röckl, 1970
189	working house painters	current skin diseases	0.10%	5 (2.8%)	Högberg and Wahlberg, 1980
3254	individuals undergoing routine patch testing	suspected allergies	0.10%	10 (0.3%)	Agren et al., 1980

Table 6. Frequency of chloroacetamide-induced contact allergy in different patient groups					
Number of individuals tested	Type of group	Pre-existing lesion	Test concentration	Number of positive tests	Reference
465	patients	eczema	0.10%	7 (1.5%)	Meynadier et al., 1982
296	patients	suspected contact allergy	0.20%	7 (2.4%)	Schulz, 1984
1081	patients	suspected contact eczema	2.0%	18 (1.7%)	Auth et al., 1984
501	patients	suspected contact dermatitis	0.2%	3 (0.6%)	de Groot et al., 1986
5202	individuals undergoing routine patch testing	suspected contact allergies	no information	17 (0.3%)	Broeckx et al., 1987
1832	patients	suspected contact allergy	no information	30 (1.6%)	COLIPA, 1987
8521	individuals undergoing routine patch testing	suspected contact allergy	no information	20 (0.25%)	Goossens et al., 1997

End of Table 6

In order to study the sensitising potency of chloroacetamide, 205 dermatologically healthy male subjects were treated with the chemical for a period of 3.5 weeks. Every 2 or 3 days, 0.5 g of the substance (1.25% chloroacetamide in ointment base) was applied to the same site on the upper lateral portion of the arm and covered with an occlusive patch for 48 or 72 hours (10 epicutaneous applications = induction phase). Following a treatment-free rest period of 2 weeks, another 1.25-percent chloroacetamide-preparation in ointment base (equivalent to 0.5 g chloroacetamide) was used in an attempt to elicit contact allergy. Skin contact was maintained for 72 hours by means of an occlusive patch and the skin reactions were then read. Of the subjects thus treated, 35 individuals, or 17% of the group, could be shown unequivocally to have developed a contact allergy. No information was given regarding the purity of the chloroacetamide used in this study (Marzulli and Maibach, 1973).

In a further study in 117 male and 33 female subjects without skin ailments, 47% of the treated individuals were demonstrated to have developed a contact allergy to chloroacetamide. During the induction phase, a 0.5-percent aqueous solution of chloroacetamide was applied 9 times under occlusive cover for periods of 48 to 72 hours. After 2 weeks, the same

solution was employed to elicit the allergic reaction by applying it to the skin of the subjects by means of an occlusive patch for two consecutive 48-hour exposures. It was apparent that the female subjects developed reactions considerably more frequently (19/33, 58%) than did the males (28/114, 25%). Again, no information was given regarding the purity of the chloroacetamide used in the study (Jordan and King, 1977).

An evaluation by the Informationsverbund Dermatologischer Kliniken (IVDK, Information Network of Departments of Dermatology for the surveillance and scientific evaluation of contact allergies in Germany) of formaldehyde allergy data from the 1992–1995 period states that of the 81 women who were definitely sensitive to formaldehyde, 5% also developed an allergy to chloroacetamide. In the males, the corresponding percentage was 9% of 34 formaldehyde allergics investigated. The data for individuals who were not sensitive to formaldehyde gave markedly lower frequencies, the percentages of chloroacetamide allergics being 1% and < 1% among the 11630 women and the 6389 men, respectively, who were included in the survey. The authors interpreted their findings as “exposure-induced combined allergies” (Schnuch and Geier, 1997).

9 Classifications and threshold limit values

In the Federal Republic of Germany, chloroacetamide has been legally classified in the TRGS 905 and placed into category R_F3 of substances toxic to reproduction (i.e. “substances which cause concern for human fertility”) in accordance with the EU classification criteria; furthermore it has been assigned the risk phrase R43 indicating sensitisation (TRGS 905, 2000).

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