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Toxicity of Chloroprene, 1,3-Dichlorobutene-2, and 1,4-Dichlorobutene-2

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A review of the toxicity of 1,3-dichlorobutene-2 (1,3-DCB), 1,4-dichlorobutene-2 (1,4-DCB), and 2-chlorobutadiene, 1,3 (β-chloroprene) was undertaken with an emphasis on assessing the hazards of these materials in the industrial situation. 1,3-DCB is a by-product in the production of β-chloroprene from the acetylene route, with 1,4-DCB is an intermediate in the production of β-chloroprene from the butadiene route, the production route used in the U. S. Presented in the review is a summary of the acute toxicity including mutagen testing, skin, eye, and inhalation testing of these compounds. In addition, subacute inhalation testing, embryotoxicity, teratogenicity, and carcinogenicity are also reviewed where the information is available.

This paper is a review of the toxicity of 2-chlorobutadiene-1,3 (β-chloroprene), 1,3-dichlorobutene-2 (1,3-DCB), and 1,4-dichlorobutene-2 (1,4-DCB).

Table 1 summarizes the interrelationship of 1,3-DCB, 1,4-DCB, and β-chloroprene. β-Chloroprene can be produced from two starting materials, either acetylene with 1,3-DCB being a major by-product or from butadiene with 1,4- and 3,4-DCB as major intermediates. For many years the acetylene route was used in the United States, but at present only the butadiene route is used. Both routes are in use in Europe and Japan, while in the U.S.S.R. only the acetylene route is used.

Table 1. Production of β-chloroprene.

Starting material	Major by-product or intermediate
Acetylene	1,3-Dichlorobutene-2
Butadiene	1,4-Dichlorobutene-2
	3,4-Dichlorobutene-1

Table 2 is a review of the toxicity of 1,3-DCB. 1,3-DCB has been tested for mutagenicity in the Ames-type system and found to be negative in *Salmonella* strains and "inconclusive" in activated yeast systems (unpublished Du Pont data). The LC_{50} in rats for a 4-hr inhalation exposure is 546 ppm ± 40. The kidney, lung, liver, spleen, and thymus were target organs following an acute inhalation exposure (*1*). 1,3-DCB is also a lacrimator and is irritating to the skin. 1,3-DCB is also reported to cause disturbance in carbohydrate metabolism and morphological changes in the peripheral blood (*2*). Repeated inhalation exposure in rats for 3–6 months, at approximately 20 ppm, showed capillary damage and effects on the kidney, liver, and spleen (*3, 4*).

Table 2. Toxicity of 1,3-dichlorobutene-2.

Test	Result
Mutagen test	Inconclusive
Single exposure (inhalation)	
Rat LC_{50} (4 hr)	546 (506-590 ppm)
	Kidney, lung, liver, spleen and thymus effects
Rabbit skin	Irritating
Rabbit eye	Lacrimator
Multiple exposure (inhalation)	
Rat (3–6 mos.) 2 ppm	No effect
Rat (3–6 mos) 20 ppm	Capillary, kidney, liver, spleen effects

Table 3 summarizes some of the toxicity information on 1,4-DCB. 1,4-DCB is a mutagen in the Ames system (unpublished Du Pont data). It is acutely

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toxic with 4-hr LC_{50} value of 86 ppm in rats (*1*). Its oral LD_{50} in rats is 89 mg/kg (*5*). The LD_{50} following skin exposure in rats is 0.62 ml/kg (*5*). 1,4-DCB is a very severe eye and skin irritant and will cause burns, particularly in the eyes, that result in irreversible eye damage (unpublished Haskell Laboratory data).

Table 3. Toxicity of 1,4-Dichlorobutene-2.

Test	Result
Mutagen	Positive
Single exposure (inhalation)	
Rat LC_{50} (4 hr)	86 (77–96) ppm Hemorrhage in lungs, liver, and spleen
Single exposure (oral)	
Rat LD_{50}	89 (41–196) mg/kg
Single exposure (skin)	
Rat LD_{50}	0.62 (0.47–0.81) ml/kg Erythema, edema
Single exposure (eye)	Severe burn, permanent damage

Table 4 shows a summary of the results of two inhalation tests, the first is a 2-week subacute exposure by inhalation at levels of 0.1 or 10 ppm in rats and hamsters. At 10 ppm in rats, the following effects were seen: growth retardation and inflammation of the respiratory tract. No effects were seen in rats at 0.1 ppm. No effects were seen in hamsters following exposure at either level in this study. In addition to the 2-week subacute, a 4-week inhalation range-finding study in rats (5 days/week, 6 hr/day) has also been conducted on 1,4-DCB at 0.5, 2, 8, and 12 ppm. The lowest effect level observed was 8 ppm. The pathologic effects of 1,4-DCB are similar to that reported in the two-week inhalation study with the major effect of the respiratory tract (unpublished Haskell Laboratory data).

Table 4. Toxicity of 1,4-dichlorobutene-2 by repeated exposure (inhalation) in the rat.

Duration	Level, ppm	Result
10 day	0.1	No effect
	10	Growth retardation, inflammation of respiratory tract; no effect on hamsters
4 weeks	0.5	No effect
	2.0	No effect
	8.0	Dose-related effects on tracheal epithelium, hematologic change
	12.0	

Table 5 summarizes the chronic data on 1,4-DCB generated by Van Duuren et al. (*6*). When 1,4-DCB was applied to the skin of mice, three times a week for a lifetime, no treatment-related increase in tumors was observed. No treatment-related increase in tumors was seen following an initiator-promotor study in mice [1,4-DCB was used as the initiator and the promotor was 7,12-dimethylbenzanthracene (DMBA)]. Intraperitoneal injections of 1,4-DCB, once weekly for a lifetime in mice, also showed no significant increase in tumors. Subcutaneous injection of 1,4-DCB, once a week for a lifetime in mice, however, did result in a slight statistical increase in tumors, with 3/30 mice showing local sarcomas at the injection site.

Table 5. Chronic studies with 1,4-dichlorobutene-2 (mice).[a]

Test	Result
Skin	
Lifetime painting 3 × weekly	No treatment-related increase in tumors
Initiator-promotor	No treatment-related increase in tumors
Intraperitoneal	
Lifetime, once weekly	No treatment-related increase in tumors
Subcutaneous	
Lifetime, once weekly	3/30 Local sarcomas

[a]Data from Van Duuren (*6*).

1,4-DCB is being studied in a 90-day inhalation study in rats and a lifetime study in rats (6 hr/day, 5 days/week). These studies are being conducted at levels of 0.5 and 5 ppm (unpublished Haskell Laboratory data).

The rest of this review will be devoted to β-chloroprene. Figure 1 shows the chloroprene generation-exposure system that was described in a paper published in 1936 by von Oettingen et al. (*7*). This 1936 paper covers quite an extensive evaluation of the toxicity of chloroprene. There are two things about this generation-exposure system that should be pointed out that are germane. First, the exposure chamber levels of β-chloroprene were not determined by analytical measurements but by weighing the cylinder before and after exposure. The weight change in the cylinder is divided by the number of hours of exposure to get the average concentration per hour. Secondly, the β-chloroprene was generated by the passage of air through the cylinder. Continuous exposure to air results in chemical change to β-chloroprene and this could cause problems in trying to assess the toxicity of chloroprene.

Table 6 shows von Oettingen's mortality data following the inhalation of β-chloroprene in rats. The air concentration values were nominal values and were not actual analytical measurements. It is obvious that a lethal level of chloroprene cannot be accu-

rately determined. At 1 mg/l, approximately 280 ppm, 25% mortality was observed; but at 6 mg/l (1680 ppm), 10 mg/l (2800 ppm), and 14.8 mg/l (4100 ppm) no mortality was observed. Looking back at these observations in light of today's knowledge, it is quite possible that these differences in mortality could be related to inaccurately determined chloroprene chamber concentrations, or more likely to change in the sample due to its age and exposure to air.

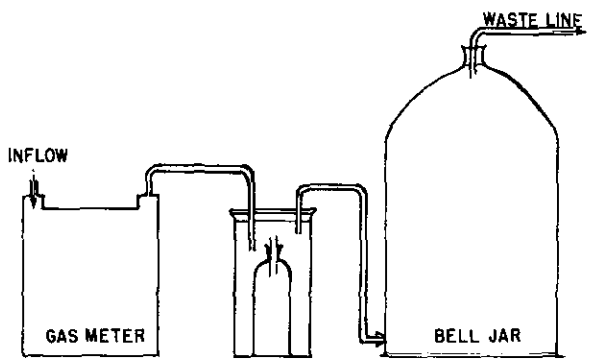

FIGURE 1: β-Chloroprene generation-exposure system (7).

Table 6. Effect of chloroprene inhalation on mortality in rats (8 hr).[a]

Concentration, mg/l.	Mortality, %
0.4	0
1.0	25
1.9	0
2.2	100
6.0	0
6.3	100
8.4	50
10.0	0
14.8	0
21.1	100

[a]Data from von Oettingen et al. (7).

Table 7 is also from von Oettingen's 1936 paper and shows the effect of chloroprene on reproduction in rats and mice. In this case, the exposed males were bred to unexposed virgin females. Here again, this same unpredictable pattern in response is observed—no indication of a detrimental effect on reproduction at certain chloroprene exposure levels, while lower levels show a definite effect on reproduction. There also appears to be a pronounced species difference. I point these data out because von Oettingen's work was done in 1936 and is the basis for much of the early knowledge of the toxicity of chloroprene. For its day, von Oettingen's work contained some very good information, and its general conclusion is still correct—chloroprene is a toxic material and should be handled with caution.

Table 7. Effect of inhalation of chloroprene on male reproduction.[a]

Rat		Mouse	
Concn, mg/l.	% of females with litter	Concn, mg/l.	% of females with litter
0.434	50	0.042	0
1.074	0	0.271	50
1.088	33	0.414	0
1.095	0	0.429	100
2.223	0	0.434	100
6.035	50	0.541	0
8.421	0		
10.000	67		
16.983	33		

[a]Data from von Oettingen et al. (7).

The next significant paper on the toxicity of chloroprene is the work reported by Nyström in 1948 (8). This paper covers some significant animal studies on chloroprene and is also a very thorough review of the published chloroprene studies up to that time. In addition to animal work, Nyström describes some human experimental work on chloroprene. There is one salient point from Nyström's animal study that requires examination.

Nyström conducted a subcutaneous acute test with two different samples, one sample which he called "fresh" chloroprene appeared to be freshly distilled, the other sample was aged or "oxidized" chloroprene. There is no description of storage conditions. The data that point out the differences in the animal's response to these two different types of chloroprene are shown in Table 8. The number of animals used (20/level) is sufficiently large to get a good evaluation. There is a definite difference in the mortality associated with the different "types" of chloroprene used.

β-Chloroprene dimerizes readily at ambient temperatures to form several cyclic compounds. The product of oxidation of β-chloroprene, even at

Table 8. Effect of chemical state of chloroprene sample on mortality in mice following subcutaneous injection.[a]

Dose, μl/g body weight	"Oxidized" % mortality	Nonoxidized % mortality
0.125	0	0
0.25	30	15
0.5	40	35
1.0	90	50
2.0	95	55
4.0	90	55
8.0	100	65

[a]Data from Nyström (8).

low temperatures, yields an unstable copolymer with which oxygen is formed by both 1,3 and 1,4 addition. The polyperoxides formed range in molecular weight up to 5000. The autoxidation of chloroprene is faster than autoxidation of dimers, so the main products of oxidation are polyperoxides (*10*). Therefore, in the manufacture of polychloroprene, β-chloroprene is freshly distilled under vacuum and is stored at −10°C under nitrogen before use. Because of its reactivity, chloroprene is normally used in an industrial situation within 24 hr after distillation.

In von Oettingen's 1936 study (*7*), no care to prevent oxidation was taken. von Oettingen's notebook has been reviewed in the hope that it could be discovered when fresh material was placed into his generation system. In this regard, no information could be found. The age or oxidized state of the chloroprene used might explain the varied response pattern seen by von Oettingen.

Table 9 shows a summary of some more recent studies on chloroprene. Chloroprene has been subjected to the Ames mutagen test several times. The first test results were negative (unpublished Du Pont data). Bartsch et al. (*11*), however, reported that chloroprene is a mutagen in *Salmonella* strain TA 100. In another more recent Ames test, β-chloroprene was positive in *Salmonella* strain TA 1535, but not in TA 100 (unpublished Du Pont data). However, chloroprene dimers, which are products of aging, are mutagens (unpublished Du Pont data). The age of oxidation state of the samples tested for mutagenicity is not known in all cases.

Table 9. Toxicity of chloroprene.

Test	Result
Mutagen	Questionable
Single exposure (inhalation)	
ALC (4 hr, rat)	2300 ppm
Single exposure (oral)	
LD_{50} (rat)	251 mg/kg
LD_{50} (mouse)	260 mg/kg
Class B poison	Negative, all routes

Some of the more recent acute toxicity data on β-chloroprene show that the approximate lethal inhalation dose of chloroprene in rats is 2,300 ppm for a 4-hr exposure (*12*). The oral LD_{50} in rats is 251 mg/kg and in mice the LD_{50} is 260 mg/kg (*13*). Chloroprene is not classified as a Class B poison by any route of exposure (unpublished Haskell Laboratory data).

Table 10 shows a summary of the conclusions of several different embryotoxicity and teratogenicity studies with β-chloroprene (*14, 15*). In the Russian studies, done at levels as low as 1 ppm chloroprene, both embryotoxic and teratogenic effects were reported. No details of how the material was generated were found nor have inquiries to the authors been productive in elucidating this information.

Table 10. Embryotoxicity and teratogenicity of β-chloroprene.

Test	Dose	Result
Inhalation (rat)		
U.S.S.R.	1 ppm	Embryo deaths, hydrocephaly, hemorrhages in fetus
U. S.	1, 10, 25 ppm	No embryotoxic or teratogenic effects
Oral (rat)		
U.S.S.R.	0.5 mg/kg	Preimplantation losses Decreased fetal weight and length

In the study by Culik et al. (*16*), pregnant rats were exposed to β-chloroprene levels of 1, 10, or 25 ppm. The experimental conditions used, as far as exposure times during gestation and length of exposure day, were exactly the same as those reported in the Russian studies. In the experiment by Culik et al. (*16*), the chloroprene was freshly distilled and kept on dry ice or in a freezer until used. Each day a new, fresh sample was used. As can be seen from Table 10, the conclusions are not consistent with the Russian studies. A preliminary, male reproduction study conducted at the same time at 25 ppm also showed no effect on male reproduction. The products of oxidation or aging of chloroprene may be a possible explanation of the difference between the U.S.S.R. and U.S. studies.

There have been two recent 4-week (6 hr/day, 5 days/week) range-finding inhalation experiments

Table 11. Repeated inhalation exposure to β-chloroprene (4 weeks, 5 days/week, 6 hr/day).

Species	Concn, ppm	Result
Rat	39	Slight growth depression and behavioral effect
	161	Loss of hair, growth retardation, liver damage
	625	Loss of hair, growth retardation, liver damage, slight effect, kidney, spleen, adrenal
Hamster	39	Slight irritation and restlessness.
	162	Growth retardation, irritation, liver damage
	627	Death

Table 12. Chronic studies (lifetime) with β-chloroprene.[a]

Route	Species Skin	Agent	Dose	Result
Skin	Mouse	Chloroprene	2/wk, 6 mo.	No tumors
		Chloroprene	2/wk, 6 mo., DMBA 5 ×	No tumors
		DMBA	2/wk, 6 mo.	92% skin tumors
Subcutaneous	Rat	Chloroprene	400 mg/kg, 10 ×	No tumors
		Chloroprene	200 mg/kg, 50 ×	No tumors
		Chloroprene	200 mg/kg, 50 × + 0.5 mg DMBA, 1 ×	57% tumors
		DMBA only	1 ×	64% tumors
Oral	Rat	Chloroprene	200 mg/kg, 2/wk, 6 mo.	No tumors

[a]Data from Zilfjan (*17*).

with β-chloroprene in rats and hamsters (*12*). The results of these experiments are summarized in Table 11. These range-finding inhalation studies were done to help select β-chloroprene levels for chronic inhalation tests in rats and hamsters following chloroprene exposure at the high level. In the hamster study, death was observed after one exposure. In the rats, mortality was also observed during the 4-week period of the test as well as severe liver and kidney effects. The animals that died showed lung changes. Rats and hamsters exposed to the mid level showed liver effects and growth retardation. Alopecia in the rats was also observed at both the mid and high level. Even at 39 ppm, the lowest level, slight growth retardation and some restlessness were observed. In general, the 39 ppm chloroprene level was assessed to be a minimum-effect level.

Table 12 shows a summary of some of the recent chronic work conducted on β-chloroprene by Zilfjan et al. (*17*). This includes a β-chloroprene skin painting study which produced no tumors and a combination of chloroprene and DMBA in a skin painting experiment in which no tumors were seen, although DMBA by itself produced a significant number of tumors following skin painting.

Zilfjan et al. (*17*) also reported that β-chloroprene does not produce tumors following subcutaneous administration. In a second subcutaneous study, the exposure to a combination of chloroprene and DMBA showed a significant tumor incidence, but the tumor rate was similar to that seen in animals treated with DMBA only.

Zilfjan et al. (*17*) also reported no tumor production in a chronic oral study with β-chloroprene. In a chronic rat inhalation study being conducted in the Netherlands, at 10 and 50 ppm chloroprene, no tumors have been seen grossly and the mortality has been negligible after one year (unpublished Du Pont data). A chronic inhalation hamster study at 10 and 50 ppm chloroprene has also been started in the same laboratory.

This review is a summary of what is known about the toxicity of 1,3-DCB, 1,4-DCB, and β-chloroprene. Several studies are ongoing, and the outcome of these studies will certainly add to our knowledge of the safety or hazards of these compounds.

NOTES ADDED IN PROOF

Recently conducted bacterial mutagen tests (Ames) at Haskell Laboratory have shown β-chloroprene to give a positive response in TA 1535 and TA 100 in both activated and nonactivated systems.

After 12 months exposure to nominal airborne concentration of 1,4-DCB of 5 ppm, there is a statistically significant increase in malignant nasal tumors in rats exposed at this level. Rats exposed at the lower level of 0.5 ppm have shown no adverse affects.

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