# Oral Toxicity Study of 1,2,2-Trichloro-1,1,2-trifluoroethane

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In the past few decades interest in the toxicity of low molecular weight chlorofluorohydrocarbons, used quite extensively as refrigerants, propellants, diluents, and solvents, has increased. Recently the use of such compounds in medicine has been investigated.[1,2] Primary interest in this regard has centered around the chlorofluoroalkanes as anesthetics. However, other uses for these compounds in medicine are possible. One such application is connected with a new concept in the treatment of gastric ulcers called "gastric freezing."[3] This procedure entails the freezing of the stomach with ethyl alcohol in place of surgery for the treatment of peptic ulcer disease. The toxicity of alcohol is such that we feel it could be replaced by safer substances possessing the desired physical properties. Many of the chlorofluorohydrocarbons possess the required physical properties, but little is known of their oral toxicity or effect upon gastric tissue. Moreover, although the inhalation toxicity of this class of compounds has been studied extensively,[4] investigations regarding oral toxicity have been limited.

In view of these facts we have conducted a preliminary investigation of the oral toxicity and effects on gastric mucosa of one representative chlorofluoroalkane. The results of our initial investigations are presented in this communication.

Acute oral toxicity of 1,2,2-trichloro-1,1,2-trifluoroethane was determined in 30 Sprague-Dawley male rats (200–300 g.) at 5 rats per dose. Twelve hours prior to intubation of the test material food only was withheld from the animals to insure uniformity as to stomach contents.

The product was administered with no added diluents. It was necessary to administer the compound in more than one dose at the higher dose levels. Such dosages were scheduled at 3-hr. intervals and the total dose administered over a period of 12 hr. Consequently, the volume administered varied from 1.0 to 12.0 ml., depending upon the dosage administered. However, no more than 4.0 ml. was administered to any animal at any one time.

Following administration of the 1,2,2-trichloro-1,1,2-trifluoroethane all animals were fed food and water *ad lib.*, housed individually, and observed for a period of 14 days. During the observation period all physical and behavioral changes as well as date and approximate time of death were noted. Autopsies for gross pathological changes in tissues and organs were performed on all animals dying during the 14-day observation period. Surviving animals were weighed and sacrificed at the end of the test period and autopsied for gross pathological changes.

Five healthy mongrel dogs were anesthetized with pentobarbital sodium (29 mg./kg., i.v.). The stomach was exposed and ligated above the cardiac sphincter and below the pyloric sphincter. A 5-cm. incision was made in the greater curvature of the stomach and a tissue sample was removed for microscopic examination (a section being removed parallel with and adjacent to the incision). The compound being tested was then introduced into the stomach and the incision closed to prevent loss of the test material. Two hours following introduction of the test chemical the stomach was opened and drained of fluid. The animals were then sacrificed and a tissue section was removed for microscopic pathological examination (a section parallel with and adjacent to the previous section, but not from the area in direct contact with the incision).

TABLE I

| Animal group | Dose, mg./kg. | Mortality, total animals | Approx. time of death | Av. wt. change at death, g. | Av. wt. change of survivors, g. |
|---|---|---|---|---|---|
| 1 | 30 | 0/5 | | ... | +46 |
| 2 | 35 | 0/5 | | ... | +41 |
| 3 | 40 | 0/5 | | ... | +19 |
| 4 | 45 | 3/5 | 5 to 24 hr. | −12 | +25 |
| 5 | 50 | 4/5 | 1 to 7 days | −49 | +31 |
| 6 | 55 | 5/5 | 3 to 9 days | 0 | ... |

Immediately following administration all animals became lethargic, their coats were ruffled, and facial edema occurred. The abdominal area was greatly distended regardless of the dosage administered. All the animals were observed to have a liquid fecal discharge. These conditions persisted for 24 hr. At the end of 48 hr. all symptoms disappeared, with the exception of the ruffled appearance which lasted, at all levels, for the duration of the study.

All of the animals dying during this experiment showed consistent gross pathological changes. Of particular significance in this respect was the observation of hemorrhage in the lungs, possibly resulting from contact of the lung tissue with the material due to its high degree of volatility. The livers of these animals also exhibited a mottled surface with normal color. The stomach and gastrointestinal tract were abnormally distended with gas and fluid. All other tissues examined appeared normal. It is interesting to note that, while those animals which died during the course of the experiment lost weight, all survivors gained weight; and that the animals at the lower levels of concentration died sooner than those at a higher concentration.

The surviving animals showed no gross pathological changes in tissues and organs except for a slight lung hemorrhaging at the higher levels. The oral $LD_{50}$ was calculated to be 43.0 ± 4.8 g./kg. by the method of Miller and Tainter.[5] When the oral $LD_{50}$ of absolute ethyl alcohol in the rat (13.6 g./kg.)[6] is compared to the oral $LD_{50}$ of 1,2,2-trichloro-1,1,2-trifluoroethane, 1,2,2-trichloro-1,1,2-trifluoroethane is found to be approximately 2.5 times less toxic than alcohol.

One male dog and one female dog were tested with absolute ethyl alcohol and one male dog and one female dog with 1,2,2-trichloro-1,1,2-trifluoroethane. One female dog was used as a control where all surgical procedures were performed but without the introduction of any material into the stomach, in order to determine if any pathological observations made were surgical in origin. The experimental results obtained in this phase of the investigation have been summarized in Table II.

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TABLE II

| Sex | Weight, kg. | Test material | Amount introduced into stomach, ml. | Amount drained from stomach, ml. | Mucosa present in stomach fluid | Gross appearance of gastric mucosa |
|---|---|---|---|---|---|---|
| Male | 20 | 1,2,2-Trichloro-1,1,2-trifluoroethane[a] | 200 | 200 | ... | Normal |
| Female | 24 | Same | 200 | 200 | ... | Normal |
| Male | 24 | Ethyl alcohol | 135 | 180 | + | Deep red in color; swollen; leathery to touch |
| Female | 16 | Same | 200 | 270 | + | Deep red in color; swollen; leathery to touch |
| Female | 20 | None | None | None | ... | Normal |

[a] Mol. wt. 187.4, b.p. 47.6°, *d* (liquid at b.p.) 1.510 g./ml., solubility, 0.009% in $H_2O$ at 21.2°.

Histological examination of the biopsy specimens indicated that no significant histological change occurred in the 1,2,2-trichloro-1,1,2-trifluoroethane-treated stomachs while marked hyperemia and edema with minimal inflammation was found in the alcohol-treated stomachs as compared to the control specimen.

## Analogs of Methyldopa and Dopa. Hydantoic Acids and Hydantoins

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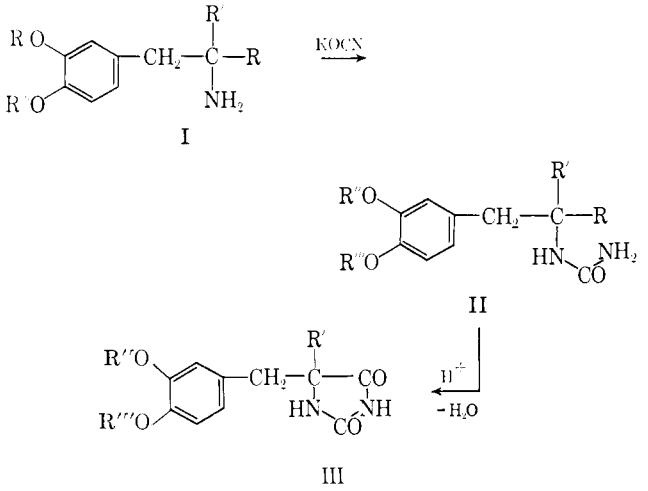
As part of a program to discover hypotensive compounds related to methyldopa (Ia), two new classes of compounds were prepared. The new analogs were 4-(3,4-dihydroxybenzyl)-4-methylhydantoic acid (IIa) and α-(4-hydroxy-3-methoxybenzyl)-α-ureidopropionitrile (IId). It had already been shown that the ability to inhibit mammalian decarboxylase was not a necessary corollary for hypotensive activity.[2,3] Thus, these analogs were not tested *in vitro* but were tested *in vivo* for their ability to depress the blood pressure of hypertensive or normotensive rats. Neither compound, however, when tested at dose levels of 100 mg./kg., i.p., showed any activity, while α-methyldopa was active in doses of 20–40 mg./kg., i.p.

Since conditions which permitted the hydrolysis of the extremely stable hydantoin led to rapid hydrolysis of the intermediate hydantoic acid to the amino acid, the desired hydantoic acids were prepared by the classical method of Dakin[4] from the amino acid. On refluxing the amino acid with potassium cyanate in aqueous solution, the salt of the hydantoic acid was obtained. The hydantoic acids were isolated after acidification with hydrochloric acid and extraction with ethyl acetate. In this manner, L(−)- and D(+)-4-(3,4-dihydroxybenzyl)-4-methylhydantoic acid (IIa) were prepared from methyldopa (Ia), L(+)-4-(3,4-dihydroxybenzyl)hydantoic acid (IIb) from dopa (Ib), and DL-4-(4-hydroxy-3-methoxybenzyl)-4-methylhydantoic acid (IIc) from the racemic 3-methoxy analog of methyldopa (Ic). DL-α-(4-hydroxy-3-methoxybenzyl)-α-ureidopropionitrile (IId) was prepared by treatment of DL-α-amino-α-(4-hydroxy-3-methoxybenzyl)propionitrile with potassium cyanate under the conditions of Herbst and Johnson.[5]

The hydantoic acids and α-ureido nitrile were readily converted into the corresponding hydantoins by refluxing in 6 *N* mineral acid. Thus, the following hydantoins were prepared: L(−)- and D(+)-5-(3,4-dihydroxybenzyl)-5-methylhydantoin (IIIa) from IIa, L(−)-5-(3,4-dihydroxybenzyl)hydantoin (IIIb) from IIb, and DL-5-(4-hydroxy-3-methoxybenzyl)-5-methylhydantoin (IIIc) from either IIc or IId.




I and II

a, R = COOH, R′ = $CH_3$, R″ = R‴ = H
b, R = COOH, R′ = R″ = R‴ = H
c, R = COOH, R′ = R″ = $CH_3$, R‴ = H
d, R = CN, R′ = R″ = $CH_3$, R‴ = H

III

a, R′ = $CH_3$, R″ = R‴ = H
b, R′ = R″ = R‴ = H
c, R′ = R″ = $CH_3$, R‴ = H

As might be expected, hydantoin formation in the methyldopa series is not accompanied by racemization, even after refluxing in 6 *N* HCl for up to 6 hr., while in the dopa series, an optically inactive product was obtained after similar treatment. Refluxing in

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