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Title
Comparison of the Acute Lethality of Selected Hydrocarbons Via Intratracheal and Oral Routes

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This report is: ☐ INTERIM ☒ FINAL and mainly: ☒ NEW ☐ REVIEW

DESCRIPTIVE SUMMARY WITH CONCLUSIONS

The approximate lethal dose (ALD) of six halogenated hydrocarbons via the intratracheal route has been determined in rats and compared with published oral LD50 values. The compounds tested in this study were dichloromethane, perchloroethylene, trichloroethylene, carbon tetrachloride, chloroform and ethylene dichloride. A method of administering the materials intratracheally to unanesthetized animals was developed. The intratracheal ALD of the halogenated hydrocarbons ranged between 3.13 and 17.5 percent of the oral LD50. Thus, aspiration of halogenated hydrocarbons may present more of a hazard than oral toxicity and should be considered when rendering first aid or emergency medical treatment.

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Study Title

Comparison of Acute Lethality of Selected Hydrocarbons via Intratracheal and Oral Routes

Authors

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Final Report

June 12, 1989

Performing Laboratory

Mammalian and Environmental Toxicology Research Laboratory
Health and Environmental Sciences
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Midland, Michigan 48674

Laboratory Project Study ID

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COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

To the best of my knowledge and belief the study described in this report was conducted in compliance with the following Good Laboratory Practice Standards

United States Environmental Protection Agency,
Title 40 Code of Federal Regulations Part 792,
Federal Register, 29 November 1983

Japan Ministry of Agriculture, Forestry and Fisheries,
59 Nohsan, Notification No. 3850,
Agricultural Production Bureau,
10 August 1984

Organization for Economic Co-Operation and Development
ISBN 92-64-12367-9, Paris 1982

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QUALITY ASSURANCE STATEMENT

STUDY TITLE: Comparison of the Acute Lethality of Selected Hydrocarbons Via Intratracheal and Oral Routes

This study was examined for conformance with Good Laboratory Practices as published by the U.S. Environmental Protection Agency, and the Food and Drug Administration. The final report was determined to be an accurate reflection of the data obtained. The dates of Quality Assurance activities on this study are listed below.

Study Start Date: 8/23/88 Date of Final Report: 6/12/89

TYPE OF AUDIT:	DATE OF AUDIT:	DATE FINDINGS REPORTED TO STUDY DIRECTOR/MANAGEMENT:
Preliminary protocol	8/09/88	8/09/88
Final protocol	9/26/88	9/26/88
Study conduct	9/26/88	9/28/88
Draft report	4/28/89	4/28/89

ARCHIVING: Raw data and a copy of the final report are filed in the testing facility archives.

T. S. Gushow 8 June 89

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SUMMARY

The approximate lethal dose (ALD) of six halogenated hydrocarbons via the intratracheal route has been determined in rats and compared with published oral LD50 values. The compounds tested in this study were dichloromethane, perchloroethylene, trichloroethylene, carbon tetrachloride, chloroform and ethylene dichloride. A method of administering the materials intratracheally to unanesthetized animals was developed. The intratracheal ALD of the halogenated hydrocarbons ranged between 3.13 and 17.5 percent of the oral LD50. Thus, aspiration of halogenated hydrocarbons may present more of a hazard than oral toxicity and should be considered when rendering first aid or emergency medical treatment.

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INTRODUCTION

The chemical pneumonitis associated with the ingestion of hydrocarbons has long been recognized. Deichmann[1] showed that hydrocarbons, administered *via* the oral or intraperitoneal routes to experimental animals, could produce pneumonitis. Gerard[2] showed that intratracheal administration of kerosene to rats also produced a pneumonitis; the amounts required being 140 times less than by the oral route. Beamon[3] reviewed 116 cases of children who had ingested various hydrocarbons and who were treated in various ways. There was a suggestion of increased incidence of pulmonary complications when emesis was induced although the difference was not statistically significant. Rumak[4] reviewed the literature and came to an opposite conclusion that induction of emesis was the best course of action; the exception being mineral seal oil (which is used in furniture polish).

Halogenated hydrocarbons are used infrequently in household materials. However, they can be found in dry cleaning fluids, some pesticide formulations, paint and varnish removers and metal cleaners such that accidental ingestion can occur. The information on the toxicity of aliphatic hydrocarbons has been gathered from clinical information in addition to animal experiments. There is data on the oral and inhalation toxicity of halogenated hydrocarbons in experimental animals but little or no human clinical data related to the effects of these materials *via* the intratracheal route. This lack of information on either humans or experimental animals *via* the intratracheal route prompted this study.

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METHODS

Animals: Sprague-Dawley rats (200-250 g) obtained from Charles River Breeding Laboratory, Portage, MI, were used in this study.

Surgery: The animals were sedated with methoxyflurane and then anesthetized with isoflurane (2.5% at a rate of 0.2 l/min) *via* a glass nose cone. A 2 cm incision was made on the ventral midline of the neck, caudal to the larynx. Adipose tissue and regional muscles were bluntly dissected to expose the trachea. Using a 17 gauge needle as a trochar, a Silastic® cannula (0.02 in id x 0.037 in od) was inserted 7 mm into the trachea. The cannula was affixed to the tissue surrounding the trachea with cyanoacrylate adhesive. A 1 cm incision was made on the dorsal aspect of the animals' neck at the same level as the cannula implantation. The cannula was tunneled subcutaneously from the implantation site to the incision on the back of the neck. Excess tubing was left subcutaneously to allow for displacement due to movement. Externally, the end of the cannula was knotted and sutured to the skin on the back of the neck. Incisions were closed with sutures. The following day, the animals had recovered fully from anesthesia and surgery.

Approximate Lethal Dose (ALD): To minimize the use of animals, the ALD of the test compounds was determined as described by Deichmann[5]. Thus, a single animal was used for each dose of the test materials and the controls. The doses were calculated on a logarithmic scale as described by Kennedy[6]. The ALD method derived the maximum amount of information using the minimum number of animals.

Dosing: The material was administered by cutting of the knot from the end of the cannula, inserting a blunt needle and injecting the

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calculated dose from a syringe. Air was used to force the test material from the cannula into the trachea. An initial test dose of 10% of the oral LD_{50}[7] was administered intratracheally. If the animal survived for the three day post-dosing observation period, the dose for the next test animal was increased by one-fourth log unit. This dosing regimen was repeated until the rat died within the three-day interval which was defined as the ALD. If the rat died following the initial dose of 10% of the oral LD_{50}, the next dose level was decreased one log unit (1% of the oral LD_{50}). As all rats survived the 1% dose level, intermediate dose levels on a one-fourth log basis were then used to determine the ALD. Control animals were prepared by implanting cannulas in the trachea. The effects of introducing fluid into the trachea were tested by injecting physiological saline at zero, one and two times the maximal volume of the test compounds.

Materials: The test compounds were as follows: methylene chloride and ethylene dichloride, (Fischer Scientific, Pittsburgh, PA, ACS grade); trichloroethylene, perchloroethylene, chloroform and carbon tetrachloride, (Aldrich Chemical Co., Milwaukee, WI, Spectrophotometric Grade).

Necropsy: All rats surviving the three-day observation period were euthanized *via* CO_2 anesthesia. A complete necropsy examination was conducted by a veterinary pathologist. Specific attention was paid to the surgical site and determination as to whether the cannula remained in the trachea. Rats dying spontaneously were similarly necropsied.

Lung changes at necropsy were summarized into three categories based on the nature of change and the extent of pulmonary parenchyma involved. These were as follows:

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Minor--Changes involved less than one-fourth of the tissue and were generally rose to dark-red foci or areas. Discolored areas were usually depressed and most likely atelectatic foci. Occasionally these foci were raised and contained fluid. As such, these most likely represented a more active inflammatory response.

Moderate--Changes involved one-fourth to one-half of the parenchyma, and were usually rose to dark-red areas. Slight pulmonary edema and fluid or froth in the trachea were frequently noted, particularly for those dying spontaneously.

Marked--Changes involved more than one-half of the parenchyma and were bright red to dark-red and frequently swollen. Pulmonary edema and tracheal fluid or froth, often pink, were usually present.

In a few cases (6), the cannula was found not to be in the trachea at necropsy; these animals were not tabulated in the study and the dose was repeated in another animal.

RESULTS

The results of this study are summarized in Table I. The ALD *via* the intratracheal route ranged between 3% and 17.5% of the published oral LD_{50}[7]. The results were analyzed statistically by the binomial probability method[8]. Since all 6 test materials were more toxic by the intratracheal route than the oral route, it was calculated that p=0.0156 which is statistically significant. Those compounds which are the most toxic orally were not necessarily most toxic by the intratracheal route.

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The findings at necropsy were variable. Control animals with no fluid injected into the cannula had no lesions or minor lung changes which indicate that the surgical procedure produces few changes. A dose of 0.89 ml/kg of saline (equivalent to the maximum volume of test compound) caused the animal to show some spontaneous movement for about 10 seconds, after which, it appeared normal. A dose of 1.77 ml/kg of saline (twice the maximum volume of test compound) produced the same reaction. Minor to moderate lesions were seen in the anteroventral lobes of saline controls.

Rats dying spontaneously generally had moderate to marked pulmonary lesions. The variability was partly due to the interval between dosing and death with rats dying peracutely generally having moderate lesions and those surviving longer having marked lesions.

Most of the animals receiving lethal doses of the test compounds died in less than ten seconds. One animal receiving carbon tetrachloride and one receiving trichloroethylene died overnight.

DISCUSSION

The results clearly indicate that halogenated hydrocarbons are much more toxic *via* the intratracheal route of administration than when given orally as are aliphatic hydrocarbons. Death in the case of aspirated aliphatic hydrocarbons is due to pneumonitis and is delayed. The rapidity with which the animals expired in this study indicates that the cause of death was absorption of the materials by the pulmonary route leading to anesthesia which rapidly progresses to Stage IV and death. When administered by inhalation, the lethal doses are anesthetic followed by respiratory arrest and circulatory collapse. Pathological changes were seen in all the animals but the most se-

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vere changes were not always seen in animals receiving the highest doses. This can be interpreted that a significant hazard from halogenated hydrocarbons is pulmonary absorption rather than the ensuing pneumonitis associated with aliphatic hydrocarbons.

Some first aid procedures have suggested that ingested halogenated hydrocarbons are so toxic that emesis should be induced to remove them as quickly as possible. The toxic manifestations are usually liver and kidney damage when these materials are given by the oral route. Other toxic materials may be dissolved in the halogenated hydrocarbons, such as pesticides, which have to be considered. Because of this toxicity, these materials should be removed from the stomach but not at the risk of aspiration into the pulmonary tract.

One also must consider that all the halogenated hydrocarbons have anesthetic properties and are capable of depressing the central nervous system. This being the case, the gag reflex may be obtunded which will increase the risk of aspiration during emesis. As pointed out by Thompson[9] "because the onset of sedation may be rapid with some poisons, the patient who is alert when emesis or lavage is begun may be stuporous and aspirating 20 minutes later, before emesis or lavage is completed. Never use emesis or lavage unless the airway is secure; aspiration is more deadly than absorption of most poisons".

The administration of milk or other fluids has been suggested in the case of halogenated hydrocarbons for the alleged purpose of dilution. Since hydrocarbons, halogenated or aliphatic, are not miscible with water or milk it is difficult to see how dilution could occur. The drinking of large quantities of fluids may promote spontaneous emesis with the risk of aspiration.

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When presented with a patient who has ingested hydrocarbons, halogenated or aliphatic, the attending medical personnel are faced with an unpleasant choice of options. If the quantity is known to be very small, the wisest course of action is probably to do nothing. In many cases, if not most, the quantity ingested is unknown. The safest course of action is probably gastric lavage after the airway is protected although this procedure is not without some hazard.

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TABLE I

COMPARISON OF THE ACUTE LETHALITY OF SELECTED HYDROCARBONS
VIA INTRATRACHEAL AND ORAL ROUTES

TEST MATERIAL	ORAL LD_{50}	DOSE % ORAL LD_{50}	IT[1] ALD	SURVIVAL[2]	PATHOLOGY[3]
CONTROL		0		+	Minor
Saline (vol)		0.89 ml/kg		+	Moderate
Saline (vol)		1.77 ml/kg		+	Minor
METHYLENE CHLORIDE	2.0 g/kg	1.0		+	Minor
		10.0		+	Marked
		17.5	0.35 g/kg	-	Moderate
		31.3		-	Marked
TRICHLOROETHYLENE	4.92 g/kg	1.0		+	Minor
		1.75		+	Moderate
		3.13	0.15 g/kg	-	Marked
		5.6		-	Marked
		10.0		-	Moderate
PERCHLOROETHYLENE	2.60 g/kg	5.5		+	Moderate
		9.6		+	Moderate
		17.2	0.45 g/kg	-	Moderate
		55.5		-	Moderate

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TABLE I (cont.)

COMPARISON OF THE ACUTE LETHALITY OF SELECTED HYDROCARBONS
VIA INTRATRACHEAL AND ORAL ROUTES

TEST MATERIAL	ORAL LD_{50}	DOSE % ORAL LD_{50}	IT ALD	SURVIVAL	PATHOLOGY
ETHYLENE DICHLORIDE	0.68 g/kg	1.0		+	Moderate
		10.0		+	Moderate
		17.5	0.12 g/kg	-	Moderate
		31.3		-	Minor
CHLOROFORM	2.00 g/kg	1.0		+	Minor
		1.75		+	Moderate
		3.13		+	Minor
		5.6	0.11 g/kg	-	Minor
		10.0		-	Moderate
CARBON TETRACHLORIDE	2.92 g/kg	1.0		+	Minor
		1.75		+	Moderate
		3.13	0.09 g/kg	-	Moderate
		10.0		-	Marked

1 IT/ALD = Intratracheal Approximate Lethal Dose
2 + indicates survival; - indicates died
3 See Appendix 1 for full discussion

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APPENDIX 1

COMPARISON OF THE ACUTE LETHALITY OF SELECTED HYDROCARBONS
VIA INTRATRACHEAL AND ORAL ROUTES
Summary of Gross Pathologic Observations

Compound	Animal ID	Dose Level (% of LD50)	Death Status	Pertinent Necropsy Observations
Control				
	88A5157		Scheduled*[1]	No lesions.
	88A5158		Scheduled	Minor lung changes in anteroventral lobes.
	88A5159	Saline- x1[a]	Scheduled	Moderate lung changes in anteroventral lobes and scattered in other portions.
	88A5160	Saline-x2[a]	Scheduled	Catheter not present in trachea. Minor changes scattered throughout lungs, primarily right side. Catheter may have been in place at the time of dosing.
Methylene chloride				
	88A5164	1%	Scheduled	Minor lung changes in an anteroventral lobe.
	88A5163	10%	Scheduled	Catheter not present in trachea. Minor lung changes in anteroventral lobes and scattered in other portions. Catheter probably not in trachea at time of dosing based upon both clinical and necropsy observations.
	88A5166	10%	Scheduled	Marked and extensive lung involvement.

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[a] Saline x1 and saline x2 indicate an intratracheal saline volume equal to or double, respectively, the largest volume of solvent given any animal. * Scheduled indicats the animal survived the 3-day observation period.

APPENDIX 1 (continued)

COMPARISON OF THE ACUTE LETHALITY OF SELECTED HYDROCARBONS VIA INTRATRACHEAL AND ORAL ROUTES
Summary of Gross Pathologic Observations

Compound	Animal ID	Dose Level (% of LD50)	Death Status	Pertinent Necropsy Observations
Methylene chloride (continued)				
	88A5167	17.5%	Scheduled	Catheter not present in trachea. Minor lung changes, primarily in the anteroventral portions. Catheter assumed not to have been in trachea at time of dosing based upon clinical and necropsy observations.
	88A5168	17.5%	Spontaneous	Moderate changes throughout lungs; primarily in the anteroventral lobes.
	88A5170	31.3%	Spontaneous	Marked lung changes involved most of the lung parenchyma.
Chloroform				
	88A5673	1%	Scheduled	Minor lung changes, primarily in the anteroventral portions.
	88A5675	1.75%	Scheduled	Moderate lung changes involved primarily the anteroventral portions.
	88A5676	3.13%	Scheduled	Minor lung changes, primarily the caudal portions.
	88A5677	5.56%	Spontaneous	Minor lung changes primarily involved the left lung lobe.
	88A5674	10%	Spontaneous	Moderate changes throughout all lung lobes.

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APPENDIX 1 (continued)

COMPARISON OF THE ACUTE LETHALITY OF SELECTED HYDROCARBONS VIA INTRATRACHEAL AND ORAL ROUTES
Summary of Gross Pathologic Observations

Compound	Animal ID	Dose Level (% of LD50)	Death Status	Pertinent Necropsy Observations
Carbon tetrachloride				
	88A5665	1%	Scheduled	Minor lung changes, primarily of the right anteroventral lobes.
	88A5667	1.75%	Scheduled	Moderate changes involved most of the lung parenchyma.
	88A5670	1.75%	Scheduled	Moderate changes involved most of the lung parenchyma.
	88A5668	3.13%	Spontaneous	Subcutaneous and muscular hemorrhages at surgical site but the catheter was in place. Moderate changes throughout the lungs.
	88A5666	10%	Spontaneous	Marked and extensive lung involvement.
Ethylene dichloride				
	88A5681	1%	Scheduled	Catheter not present either externally or internally. Moderate lung changes involved primarily the anteroventral lobes. Based on the lung changes, the catheter was assumed to be in place at the time the rat was dosed.
	88A5682	10%	Scheduled	Moderate lung changes present, primarily at the hilus of the lung lobes.
	88A5683	17.5%	Spontaneous	Moderate lung changes involved primarily the areas near the hilus of the lobes.
	88A5684	31.3%	Spontaneous	Minor lung changes throughout all lobes.

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APPENDIX 1 (continued)

COMPARISON OF THE ACUTE LETHALITY OF SELECTED HYDROCARBONS VIA INTRATRACHEAL AND ORAL ROUTES
Summary of Gross Pathologic Observations

Compound	Animal ID	Dose Level (% of LD50)	Death Status	Pertinent Necropsy Observations
Trichloroethylene				
	88A5177	1%	Scheduled	Catheter not present either externally or internally. Minor lung changes, primarily in the anteroventral portions. Catheter may have been in place at time of dosing.
	88A5183	1.75%	Scheduled	Moderate lung changes involved the entire left lobe and the anteroventral portions of the right lobes.
	88A5184	3.13%	Spontaneous	Marked changes throughout the entire lungs.
	88A5185	5.6%	Spontaneous	Marked changes involved much of the lungs, particularly near the hilus.
	88A5180	10%	Scheduled	Catheter not in place. No lung lesions were noted. It is assumed that the catheter was not in place when the animal was dosed.
	88A5182	10%	Spontaneous	Moderate changes involved all lung lobes, particularly near the hilus.
Perchloroethylene				
	88A5172	1%	Scheduled	Moderate lung changes in all lobes, particularly the anteroventral portions.
	88A5173	1.75%	Scheduled	Moderate lung changes present throughout the lungs, particularly the anteroventral portions.
	88A5174	3.13%	Spontaneous	Moderate lung changes in all lung lobes.
	88A5171	10%	Spontaneous	Moderate lung changes in all lung lobes, especially near the hilus.

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