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THE TOXICITY AND POTENTIAL DANGERS OF INERTEEN

Submitted by

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EXHIBIT
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The Toxicity and Potential Dangers of Inerteen and Related Substances, Including a Method of Analysis for Halogenated Hydrocarbons in Air.

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The toxicological experiments on this problem were carried out by Dr. J. H. Foulger and Dr. A. J. Fleming of the Department of Toxicology and the pathological studies were made by Dr. W. T. Read, Jr. of the Department of Pathology of the Haskell Laborato

The material was investigated with oral administration to rats, application to the skin of rats, and in inhalation experiments. The latter were planned in such a way as to approach as closely as possible the actual conditions encountered in the use of this material in transformers, in order to evaluate the maxim hazard which may be encountered in its use for this purpose. Fo comparison, additional tests were made regards the toxic effects from inhalation of vapors from Pyranol and Transformer Oil (West inghouse). In addition, a method was worked out for the determination of Inerteen in air.

Effect of Oral Administration.

In order to determine the minimal fatal concentration of Inerteen for rats, 100 animals were given by stomach tube single doses of Inerteen, as indicated in the table.

Dose cc./kgm.	No. of animals treated	No. of dead	Time of death	Per cent mortality
1.0-1.49	26	5	4 + within 3 days	19.2
1.5-1.99	24	9	6 + " 2 "	37.5
2.0-2.49	10	3	3 + " 3 "	30.0
2.5-2.99	20	10	9 + " 2 "	50.0
3.0-3.49	20	15	15 + " 3 "	75.0

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In these, as in subsequent experiments, all rats showed signs of discomfort immediately following the administration, later they became depressed and refused, frequently, water and food; within a few hours they became weak and stuperous, so that they were finally unable to rise. When this stage was reached they invariably died. One of these rats was found to suffer from albuminuria, one developed tremors of the head and fore-limbs, and one showed severe hemorrhages from the intestinal tract. The table indicates that with oral administration the minimal fatal dose of Inertech for rats, which kills 75 per cent of the animals within 5 days, is 8.0 to 2.49 cc. per kilogram body weight.

Upon pathological examination of 20 of these animals, those that had died spontaneously showed congestion of the lungs, which was combined with edema and hemorrhage in several instances. Ten of the animals had mottled gray or yellow livers and in three instances there was a diffuse pallor with moderate yellowness. In 5 out of 6 animals studied microscopically the liver showed marked and rather extensive hyaline necrosis in the central part of the lobules; the liver cells in this area revealed fatty degeneration of the cytoplasm; and in 2 animals that died 52 and 54 hours after the treatment these cells contained increased numbers of mitotic figures. In the majority of the animals the mucosa of the intestine was congested and the fecal material in the intestines contained old blood; and in one instance fresh blood was found in the lower third of the ileum, lower colon,

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and sigmoid. Microscopically the stomach of two animals presented typical hemorrhagic erosions of the mucosa; another showed hemorrhagic areas in the mucosa; and in the remaining animals postmortem changes did not allow an exact investigation.

In order to follow up the toxic effect of Inerteen on the liver, a group of 30 white rats was treated in the same way with single oral doses of 1.15 to 1.20 cc. per kilogram body weight. Six of these were killed 3, 8, and 11 days, respectively, after the administration.

Upon pathological examination it was found that two of the 6 animals killed after 3 days had bloody materials in their stomachs and in 50 per cent of these animals the liver was slightly yellow in color and the liver cells were large and contained numerous mitoses and occasionally there was necrosis of cells immediately about the central veins. Animals killed after 8 days presented a slight enlargement of the liver with a questionable yellowish color, the liver cells were large, those arou the central veins were dark, and occasionally there was some fatty degeneration of these cells. Rats killed after 11 days showed no important gross pathology. Microscopically, all sections showed a slight swelling of the liver cells with occasiona vacuolization of the cytoplasm. No other organs showed patholo changes referable to the exposure.

In order to study the effect of repeated oral administrati of Inerteen, 3 groups of 10 rats each were treated in the follo ing way:

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The first group received from 0.85 to 0.86 cc. per kilo for 3 consecutive days. None of them died and 5 were killed after the 3rd treatment for pathological examination. The second group was fed doses from 0.83 to 0.88 cc. per kilo. Two died following the 2nd dose and 5 were killed for autopsy after the 3rd administration. In the third group the doses ranged from 1.23 to 1.78 cc. per kilo. One rat died after the 1st, three after the 2nd, and four after the 3rd treatment, and the remaining animals were killed four days after the 3rd treatment.

Upon pathological examination the livers of the rats of the first group appeared somewhat lighter than usual and one showed large yellow areas; there was a diffuse mild swelling of the liver cells with some polychromacia; occasionally the cells show some vacuolar changes of the cytoplasm, but in general these changes were not very severe. In 4 rats of the third group whic died after 3 treatments the stomachs were dilated with food and in two of these there was also some old blood present. Microscopically, one rat had small inflammatory ulcers in the fore stomach and another showed hemorrhagic erosions of the pylorus. All showed some hyperemia of the liver and in one rat this was of mottled appearance. Microscopically two rats showed swelling of the liver cells with fatty degeneration of those in the centr parts of the liver lobules. Two others presented central necros of the liver cells with hyperplasia of the endothelial cells in these areas, one showing in addition, numerous mitotic figures i

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the liver cells. Of 2 rats which were killed 4 days following the 3rd administration, one had a small amount of blood in the gastric content. The livers of both animals appeared moderately enlarged and microscopically there was a generalised swelling of the liver cells with some polychromasia. One rat suffered from a fairly general vacuolar degeneration of the liver cells and another presented vacuolar changes in the central part of the lobules.

In order to compare the toxic effects of Inerteen with trichlorobenzene and ethyl-tetra-penta-chlorobenzene, a group of 10 rats was given daily doses of trichlorobenzene ranging from 0.36 to 0.58 cc. per kilo body weight. One died after the 1st, four after the 2nd and four after the 3rd administration, the remaining rat was killed 4 days following the 3rd and last treatment.

It appears, therefore, that daily doses of 0.36 to 0.58 cc. per kilo of trichlorobenzene killed 80 per cent of the animals with 3 doses, whereas it was found that doses of 0.62 to 1.04 cc per kilo of ethyl-tetra-penta-chlorobenzene, given in the same way, killed only 10 per cent, and Inerteen in doses of 1.42 to 1.78 cc. per kilo, given in the same way, killed 80 per cent of the rats.

Upon pathological examination, animals which were treated with trichlorobenzene and which died spontaneously showed a passive congestion of the lungs and the liver; the stomachs were distended with food; in four some blood was present in the

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gastric contents and in one instance there was a hemorrhagic erosion of the mucosa. In two rats the liver showed irregular mottling. Liver sections of all animals showed necrosis of the liver cells around the central veins and those in the outer parts showed vacuolar degeneration. In rats which died 3 to 4 days after the first treatment there was some endothelial hyperplasia in the areas of necrotic liver cells. One animal presented an increase in the number of mitotic figures in the liver cells.

Of the 7 rats treated with ethyl-tetra-penta-chlorobenzene, the one which died spontaneously after the 3rd dose showed congestion and hemorrhages of the lungs, generalized passive congestion of the spleen, bone marrow and other viscera, distended stomach, and moderate yellow mottling of the liver, the liver cells being mildly swollen and showing fine vacuolar degeneration. The animals which were killed showed only a questionable enlargement of the liver and kidney and a slight swelling of the liver cells with occasional vacuolar degeneration, which changes were extremely mild.

It appears, therefore, that Inerteen is considerably less toxic than trichlorobenzene, the minimal fatal dose with oral administration, killing 70 per cent of the animals, being around 2.0 cc. per kilo rat; whereas ethyl-tetra-penta-chlorobenzene does not appear to be nearly as toxic with oral administration. The pathological changes observed after oral administration of Inerteen appear to be fairly consistent and obvious. Large doses

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which are fatal in one or two days, cause terminal passive congestion of the viscera, extensive degeneration of the liver with necrosis around the veins, and locally, ulceration of the gastric mucosa. Whereas sublethal doses cause within three days gastric irritation and hepatic degeneration with signs of regeneration, the latter is decidedly more marked after eight days, and after eleven days no definite liver pathology may be present. It should also be pointed out that single large doses appear to be more injurious than such doses given in fractions.

Effect of Application to the Skin.

Ten white rats were treated daily with an application of about 0.5 cc. of Inerteen to the shaved skin of the back, precautions being taken to prevent absorption through the gastro-intestinal tract by licking and by inhalation. All rats lost weight during the experiment but they showed no toxic signs aside from a slight restlessness at the beginning of the experiment. At the site of the administration the skin became dry, thickened and scaled without any fissures being formed. Similar reactions were observed on the human skin especially on the hands, cheeks and forehead. One of the rats died after 11, and three after 13 treatments, the remaining being killed 24 hours after the 18th application.

Upon pathological examination the liver of all animals was found to be dark gray brown in color with mottling on the

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surface. In those rats which died spontaneously there was fatty degeneration of the liver cells with necrosis of those around the central veins and in animals killed at the end of the experiment there was a mild swelling of the liver cells with occasional vacuolar degeneration in some. Microscopically the skin at the site of application of 4 rats showed hyperkeratinization with occasional blister formation, one animal showed perifolliculitis, one hyperplasia of the epithelium rather than hyperkeratinization, and in 3 rats there was a mild fibroblastic proliferation with some round cell infiltration in the underlying connective tissue.

It appears, therefore, that the contact of Inerteen with the skin does not only cause local reactions such as dryness, thickening and scaling of the skin, but also systemic effects as indicated by the injurious effect on the liver.

Effect of Inhalation of Vapors.

In evaluating the toxic effects of inhalation of vapors liberated from Inerteen at elevated temperatures, attempts were made to reproduce conditions more severe than would be met in the overheating of a transformer. In these experiments rats were exposed in a limited space of about 1 cubic foot capacity to the vapors of the boiling material which entered directly into the chamber through a hole in the bottom. In each experiment 8 rats were exposed on 58 to 66 occasions for 30 minutes, twice daily, to vapors from Inerteen, Pyranol, or

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Transformer Oil (Westinghouse). During this time they showed irritation of the skin, which appeared to be most marked on those parts of the body not protected by hair, and which persisted for some time after the exposure. Pyranol appeared to produce the most effect in this respect, followed by Inerteen and Transformer Oil, in the order given. In opposition to the 8 rats exposed to Pyranol and Transformer Oil, 3 out of 6 rats exposed to Inerteen lost weight, but no other toxic signs were observed. It is quite possible that rats exposed in this way absorbed some toxic material through the gastro-intestinal tract by licking the fur during or after the exposure since the material had a tendency to condense on the walls of the chamber and on the surface of the animals. The maximum concentration of Inerteen to which these rats were exposed was determined as about 0.5 mg. per liter of air.

Upon pathological examination of rats exposed in this way on 14, 35 and 66 occasions to Inerteen, no definite pathological signs could be observed in the liver or other organs which could be referred to the exposure.

Two rats treated with 41 exposures to the vapors of Pyranol showed no definite cellular changes in the liver although in one animal there was an increased number of mitotic figures in the liver cells.

Two rats exposed on 44 occasions to the vapors of Transformer Oil (Westinghouse) showed no definite pathological changes of the internal organs.

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In order to study the effects of continued exposure, 10 rats were exposed 8 hours daily on 5 days a week for 20 days to concentrations of 0.05 mg. to 0.09 mg. of Inerteen per liter of air. They showed no toxic nor pathological signs referable to the exposure; there were no definite changes of the red blood corpuscles or the hemoglobin. The animals were killed 2 days after 20 exposures.

It appears, therefore, that neither frequently repeated short exposures for 20 minutes each to concentrations of 0.5 mg. of Inerteen per liter air, nor continued exposure to concentrations of 0.05 to 0.09 mg. per liter air cause a definite toxic effect on the liver.

Determination of Concentrations of Inerteen in the Air.

In order to determine the concentration of Inerteen vapors in the air, the following nephelometric method was worked out, after several other procedures had been considered.

A definite volume of air to be tested is drawn at a rate of about 1 liter every 4 minutes through a test tube containing 5 cc. of ethyl alcohol, immersed in dry ice-acetone mixture in order to prevent the evaporation of the alcohol. At the end of the sampling 1 cc. of the alcohol was mixed with 10 cc. of distilled water and the resulting turbidity was measured with a photo electric cell, using a sodium vapor lamp as the source of light.

The turbidity of unknown samples was compared to the

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These experiments show that the concentrations of Inerteen in air decrease rapidly with the distance from the source of their production.

In order to study variations of the concentrations over a period of time and with different temperatures, a current of 10 liters per minute of air was passed over the surface of Inerteen of about 10 square inches, contained in a flask and thence into a bell jar. Samples were taken from the jar at various intervals after the flow was started and with various temperatures. The following concentrations were determined:

Time of sampling in hours	Concentration of trichlorobenzene in mg. per liter	Temperature of trichlorobenzene in flask °C.
	Trichlorobenzene	
½	0.29	23
2	0.51	23
6	0.60	23
1	1.68	50
4	1.70	50
1	1.80	90
	Inerteen	
6	0.58	23

This tabulation shows that the determinations made at 23°C. and with the 6 hour sample were almost the same for trichlorobenzene (0.60 mg. per liter) and Inerteen (0.58 mg. per liter). This suggests the possibility that the bulk of the vapors liberated from Inerteen at this temperature may be trichlorobenzene; so that the hazards from inhalation of Inerteen vapors are identical with those from the inhalation of

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trichlorobenzene. In order to confirm this, 835 cc. of Inerteen were submitted to fractional distillation and the following results were obtained:

Fraction	Temperature of distillation °C.	Volume collected cc.	Specific Gravity of fraction	Boiling Point °C.
1	240	35	1.469	208-219
2	240-254	45	1.478	228
3	254-280	45	1.493	240
4	280-300	30	1.577	269
Residue	above 300	180	1.592	-

Since the specific gravity of trichlorobenzene is 1.466 and the boiling point 208°C.-219°C., it is evident that up to 240°C. the vapors consist exclusively of trichlorobenzene and that for this reason the above statement is justified, namely, that for most conditions the hazards from vapors of Inerteen are identical with those from trichlorobenzene.

Résumé.

The experiments discussed in this report show that with oral administration Inerteen is considerably less toxic than trichlorobenzene, the minimal fatal dose killing from 70 to 75 per cent of the rats being around 3.0 cc. per kilogram body weight and 1.4 to 1.8 per kilogram body weight, respectively. Fatal doses will cause passive congestion of all viscera, extensive degeneration of the liver, and ulceration of the gastric mucosa. With sublethal doses these effects are less severe and there is evidence that they will heal when no further exposure occurs.

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Continued application of Inerteen to the skin of rats and humans causes dryness of the skin, thickening and scaling; and the observations made in rats indicate that sufficient quantities may be absorbed through the skin to produce injurious effects on the liver.

Repeated inhalation for short periods of concentrations of 0.5 mg. of Inerteen vapors per liter of air, or repeated inhalation for 8 hours of concentrations of 0.05 to 0.08 mg. per liter of air causes no definite injurious effects in rats. Vapors liberated from boiling Pyranol, Inerteen, and Transformer Oil (Westinghouse) are irritant and the irritant action decreases from Pyranol to Transformer Oil (Westinghouse), Inerteen being intermediate in this respect.

It has been shown that vapors liberated from Inerteen under conditions approaching those as might be encountered in transformers consist mainly of trichlorobenzene.

A simple method for the determination of Inerteen (trichlorobenzene) vapors in air is given. If 10 liters of air are drawn at a rate of about 1 liter every 2 minutes through a test tube containing 1 cc. of ethyl alcohol, cooled in dry ice or in an ice salt mixture, and the alcohol is then poured carefully on the surface of a few cubic centimeters of water in a test tube, a ring of turbidity is produced if the concentration of Inerteen (trichlorobenzene) is over 0.03 mg. per liter of air. If under these conditions a white ring is produced,

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the air tested should be regarded with suspicion and should be improved by more effective ventilation.

It was found that the concentration of Inerteen (trichlorobenzene) in a closed space 14 inches above the surface of boiling Inerteen is 4.8 mg. per liter and in an open space 5 inches above the surface of boiling Inerteen is 0.56 mg. per liter, and when the temperature in a transformer, standing in a closed room (vault), rises to 90°C. the air may contain 1.9 mg. per liter of air.

From the animal experiments reported, it appears that concentrations below 0.05 mg. per liter of air will cause no toxic signs or symptoms even with continued exposure.

Regards preventive measures, the following may be suggested

It appears to be of paramount importance that the contamination of the air be reduced to concentrations below 0.05 mg. per liter of air by adequate forced ventilation at the site of the production of such vapors.

Greatest personal hygiene is not less important. Contamination of the skin should be avoided by wearing proper protective garments such as gloves, caps, and coveralls. In case of acciden or with exposure to higher concentrations of and above 0.2 mg. pe liter, respirators or open air masks should be worn. The skin should be kept immaculately clean and ointments such as lanolin or Aquafor should be applied to the skin after washing the hands.

In view of the hepatotoxic action of this compound, individuals suffering from injuries of the liver, syphilis, and heart

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diseases should be excluded from operations in which Inerteen is handled.

Workers handling Inerteen or with frequent exposure to its vapors should have periodic examinations, special attention should be paid to their nutritional condition, and the condition of the liver may be checked by determining the icteric index in the blood, and the urobilin and urobilinogen excretion in the urine.

Regards the <u>treatment of toxic effects</u> produced by exposure to Inerteen, it appears advisable to increase the resistance of the liver by carbohydrate diet; administration of glucose and insulin may be advisable, and duodenal lavage with magnesium sulfate may be tried. Local irritation of the skin produced by Inerteen should be treated with bland ointments after the toxic material has been removed by intensive washing and scrubbing. In case Inerteen has been taken by mouth, the toxic material should be removed from the stomach by emetics such as soap water or mustard water, and this should be followed by gastric lavage with a suspension of activated charcoal in water and saline cathartics such as Epsom salts.

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