

Medical Research Project No. MR-45.

THE TOXICITY AND POTENTIAL DANGERS OF INERTEN

Submitted by

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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. E. Foulger and Dr. A. J. Fleming of the Department of Toxicology and the pathological studies were made by Dr. W. Read, Jr. of the Department of Pathology of the Haskell Laboratory.

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 closely as possible the actual conditions encountered in the use of this material in transformers, in order to evaluate the hazard which may be encountered in its use for this purpose. In comparison, additional tests were made regarding the toxic effect from inhalation of vapors from Pyranol and Transformer Oil (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 5 doses of Inerteen, as indicated in the table.

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Dose cc./kgm.	No. of animals treated	No. of dead	Time of death	Per cent mortality
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1.0-1.49	26	5	4 $\frac{1}{2}$ within 3 days	19.
1.5-1.99	24	9	6 $\frac{1}{2}$ " 3 "	37.
2.0-2.49	10	3	3 $\frac{1}{2}$ " 3 "	30.
2.5-2.99	20	10	9 $\frac{1}{2}$ " 3 "	50.
3.0-3.49	20	15	15 $\frac{1}{2}$ " 3 "	75.

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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 stuporous, 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 Inerteen for rats, which kills 75 percent of the animals within 3 days, is 3.0 to 2.49 cc. per kilo gram 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. 10 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 portions of the lobules; the liver cells in this area revealed fatty degeneration of the cytoplasm; and in 2 animals that died 32 and 34 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 8 days presented a slight enlargement of the liver with a questionable yellowish color, the liver cells were large, those at 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 occasional vacuolization of the cytoplasm. No other organs showed pathological changes referable to the exposure.

In order to study the effect of repeated oral administration of Inerteen, 3 groups of 10 rats each were treated in the following ways:

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The first group received from 0.25 to 0.56 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 0.63 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 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 polychromasia; occasionally the cells showed some vacuolar changes of the cytoplasm, but in general these changes were not very severe. In 4 rats of the third group died after 3 treatments the stomachs were dilated with food in two of these there was also some old blood present. Microscopically, one rat had small inflammatory ulcers in the forestomach 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 the rats showed swelling of the liver cells with fatty degeneration of those in the parts of the liver lobules. Two others presented central necrosis of the liver cells with hyperplasia of the endothelial cells in these areas, one showing in addition, numerous mitotic figures.

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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 generalized swelling of the liver cells with some polychromasia. One rat suffered from a fairly general vacuolar degeneration of the liver cell 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 1st, four after the 2nd and four after the 3rd administration; the remaining rat was killed 4 days following the 3rd and 1st treatment.

It appears, therefore, that daily doses of 0.36 to 0.5 per kilo of trichlorobenzene killed 80 per cent of the animals with 3 doses, whereas it was found that doses of 0.62 to 1.0 per kilo of ethyl-tetra-penta-chlorobenzene, given in the same way, killed only 10 per cent, and Inerteen in doses of 1.21 to 1.72 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 passive congestion of the lungs and the liver; the stomach were distended with food; in four some blood was present in

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 more toxic than trichlorobenzene, the minimal fatal dose with oral administration, killing 75 per cent of the animals, being 3.0 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

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which are fatal in 72 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 gastrointestinal 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 at 11, and three after 13 treatments, the remaining being killed 24 hours after the 15th application.

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

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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 fibroblast 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 effect 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 2 rats were exposed on 15 to 66 occasions 10 minutes, twice daily, to vapors from Inerteen, Pyranol,

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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 2 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 0.5 mg. per liter of air.

Upon pathological examination of rats exposed in this on 14, 25 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 P. showed no definite cellular changes in the liver although in 1 animal there was an increased number of mitotic figures in 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 vap 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 the sampling 1 cc. of the alcohol was mixed with 10 cc. of distilled water and the resulting turbidity was measured with photo electric cell, using a sodium vapor lamp as the source 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.
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Trichlorobenzene

3	0.29	23
2	0.51	23
6	0.60	23
1	1.68	50
4	1.70	50
1	1.90	90

Inerteen

6	0.58	23
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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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turbidity produced by known standards made up of trichlorobenzene and ethyl alcohol. This method gave satisfactory readings over a range from 0.80 to 8.0 mg. per liter of air and when the original dilutions were taken into account, it was sufficiently sensitive to detect quantities as low as 0.03 mg. of Inerteen per liter of air. The method is not specific for the various members of the halogenated hydrocarbons but since the vapors liberated from Inerteen consist mainly of trichlorobenzenes, as will be shown below, it may be used for the determination of Inerteen vapors in air.

In order to obtain information regards changes of the concentration of vapors in air with the distance from the source of production, samples of air were taken in a closed space at varying distances from boiling Inerteen and the following concentrations were determined:

Distance (inches)	Concentration mg. per liter
14	4.50
20	1.88
28	1.14
36	1.14

When samples were taken at various distances over an open beaker of boiling Inerteen the results were as follows:

Distance (inches)	Concentration mg. per liter
5	0.56
10	0.42
15	0.24
20	0.24

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trichlorobenzene. In order to confirm this, 235 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	38	1.468	208-219
2	240-254	48	1.478	228
3	254-280	43	1.493	240
4	280-300	30	1.877	269
Residue	above 300	180	1.892	-

Since the specific gravity of trichlorobenzene is 1.486 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 there is evidence that they will heal when no further exposure occurs.

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 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 0.05 mg. per li of air. If under these conditions a white ring is produced

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 8 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 1.2 : 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 contact or with exposure to higher concentrations of and above 0.2 mg. per liter, respirators or open air masks should be worn. The skin should be kept immaculately clean and ointments such as lanolin or Aquaford should be applied to the skin after washing the body.

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

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 treatment of toxic effects 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 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.

THE TOXICOLOGY OF INERTEN AND RELATED SUB-
STANCES INCLUDING A METHOD OF ANALYSIS FOR
HALOGENATED HYDROCARBONS IN THE AIR.

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The Toxicology of Inerten was investigated by oral administration, skin application, and inhalation experiments in rats. In the latter an attempt was made to investigate the substance in a way relative to its use in transformers and to determine the maximum hazard that might arise should the substance be used for that purpose.

The minimum fatal dose was determined by oral administration and the effect of repeated oral doses was studied and compared with the effect of similar doses of trichlorobenzene and ethyl-tetra-penta-chlorobenzene.

Ethyl-tetra-chlorobenzene
A method of air analysis for the halogenated hydrocarbons was devised and air analyses were made to determine the concentrations of Inerten in the air near open and closed containers when the substance was heated to various temperatures and when samples were taken at various distances from the source. A statement of the problems incident to the toxicology of Inerten and the answers to these problems will be found in conclusions and summary at the end of the paper.

Inerten

Oral Administration:

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(1). Single Doses.

The substance was given in single graded doses to several groups of rats to determine the minimum fatal dose.

TABLE I

MINIMUM FATAL DOSE OF INKTEEN WITH
SINGLE ORAL ADMINISTRATION

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<u>Dose mg./Kilo</u>	<u>No. of Animals Treated</u>	<u>No. of Deaths</u>	<u>Time of Deaths</u>	<u>Percent Mortality</u>
1.00 - 1.49	26	5	4 died within 3 days	19.2
1.50 - 1.99	24	9	6 " " 3 "	37.5
2.00 - 2.49	10	3	3 " " 3 "	30.0
2.50 - 2.99	20	10	9 " " 3 "	50.0
3.00 - 3.49	20	15	15 " " 3 "	75.0

The results are conveniently tabulated in table 1.

Table 1

As noted, a dose of 3 to 3.5 cc. per kilogram gave a 75 per cent mortality. Twenty-two of this group of animals were autopsied. Another group of 33 rats was given single oral doses ranging from 1.15 to 1.20 cc. per kilogram. Six of these were killed 3 days after treatment and examined by the pathology department for possible liver damage. Six were killed 8 days after treatment and examined for possible kidney change and 6 were killed 11 days after treatment, and examined for possible brain damage.

(2) Repeated Doses

Thirty rats were divided into groups of ten. The first group received from 0.35 to 0.55 cc. per kilogram for 3 treatments. None died and 5 were killed for autopsy within 24 hours after the 3rd treatment. In the second group, the dosage ranged from 0.63 to 0.88 cc. per kilogram. Two died after the second treatment and 5 were killed for autopsy 24 hours after the last treatment. In the third group the dosage ranged from 1.23 to 1.78 cc. per kilo. One died after 1 treatment, 3 died after 2 treatments, and 4 died after the 3rd treatment. All animals in this last group were autopsied within 24 hrs. of the last treatment.

In general all rats behaved the same following oral administration of Inertsen. They proceeded at once to eat as if to allay the discomfort caused by the substance. Following this they became depressed and would not eat and often would :

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drink. Weakness and stupor generally supervened within a few hours until the animals could no longer rise for food and water. Liquids were taken, however, if administered and for a few minutes following administration the animals perked up a bit. The improvement was only temporary and the outcome, when they had thus far progressed, was invariably fatal. One animal showed an albuminuria (tested post mortem) and one developed tremors of the head and front limbs. One had severe hemorrhages from the rectum and the autopsy indicated the bleeding was from the lower end of the small bowel.

Comparison of Inerteen with Trichlorobenzene and Ethyl-tetra-penta-chlorobenzene

A group of 10 animals was given repeated doses of trichlorobenzene ranging from 0.36 to 0.58 cc. per kilogram. One died after the 1st treatment, 4 died after the 2nd, and 4 died after the 3rd. The remaining animal was killed 4 days after the 3rd and last treatment. All animals were autopsied. Similar groups were treated with Inerteen and ethyl-tetra-penta-chlorobenzene and the following noted:

Daily doses of 1.23 to 1.76 cc. per kilogram of Inerteen killed 80 per cent of the animals, when 3 doses were given, while daily doses of 0.36 to 0.58 cc. per kilogram of trichlorobenzene killed 90 per cent of the animals with 3 doses. On the other hand daily doses of ethyl-tetra-penta-chlorobenzene ranging from 0.62 to 1.04 cc. per kilogram killed 1 rat in a group of 10 with 3 doses.

Conclusion:

The minimum fatal dose of Inerteen appears to be

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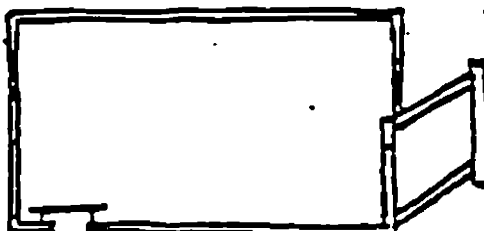
around 3.0 cc. per kilogram. Repeated doses of Inerteen were fatal in concentrations between 0.86 and 1.78 cc. per kilogram, while repeated doses of trichlorobenzene were fatal in concentrations between 0.36 and 0.58 cc. per kilogram. Ethyl-tetra-penta-chlorobenzene does not appear to be nearly so toxic by oral administration.

Skin Exposure:

Half a cubic centimeter of Inerteen was applied daily to the shaved backs of 10 rats by means of a small glass cup filled with a piece of cotton wool soaked in the substance. The whole was strapped to the animals with adhesive tape. One rat died after 11 treatments. Three died after 13 treatments and remaining animals were killed for autopsy 24 hours after the 1 treatment. All rats lost weight during the experiment, but outside of a slight restlessness at the beginning of the experiment showed no other signs. The Inerteen acted locally on the skin producing an excessive dryness with subsequent thickening and scaling without any fissures being formed. The same effects were noted on the human skin with respect to the drying and slight scaling and were most notable on the hands, cheeks and forehead.

Inhalation

An attempt was made to reproduce conditions more so than would be met in the overheating of a transformer and exposing rats to these conditions for 20 minutes twice a day.



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The foregoing diagram represents a box of about 1 cubic foot capacity, in one corner of which is the inlet from a glass flask containing the substance being investigated. Inerteen, Pyranol or transformer oil (Westinghouse) were placed in the flask and brought to a boil. Two rats were then placed in the box and allowed to remain for 20 minutes, during which time a generalized itching of the skin appeared which was most marked in the parts of the body not protected by hair. The itchiness persisted for sometime after the animals were removed from the box. Pyranol seemed to produce the most effect in this respect with Inerteen and Transformer oil following in the order named. Three out of 6 of the Inerteen treated rats lost weight, while the Pyranol and Transformer oil treated rats gained weight (2 rats only in each of the last two groups). It is quite possible that these animals ingested a considerable amount of these substances by licking their fur after removal from the box. No marked change in respirations were noted, although the animals once or twice breathed faster than normal and occasionally the extremities were slightly blue. At autopsy no gross pathology was noted. The maximum concentration of Inerteen to which these rats were exposed was about 0.5 mg. per liter. The box was aired between readings, but not washed out.

With Inerteen two rats received 28 treatments, two received 33 treatments and two received 66 treatments. With Pyranol two rats received 42 treatments and with Transformer Oil two rats received 44 treatments.

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A group of ten rats was exposed for 20 days, eight hours per day to concentrations of Inerteen in the air ranging from 0.03 to 0.09 mg. per liter. All rats were killed for pathology within two days following the 20th days of treatment. Eight of this group gained weight, one lost weight and one showed no change. Five showed a slight drop in the red blood count, three showed an increased red blood count and two showed no change. The changes in the hemoglobin paralleled the changes in the red blood count.

Air Analysis for Halogenated Hydrocarbonated Hydrocarbons.

Considerable difficulty was experienced in obtaining a satisfactory method of air analysis for these substances and a nephelometric method was finally adopted. In this, a definite volume of the air to be tested was drawn at a rate of about 1 liter every four minutes through 5 cc. of ethyl alcohol cooled in a dry ice-acetone mixture. The latter being necessary to prevent evaporation of the alcohol. One cubic centimeter of the alcohol was then mixed with 10 cc. of distilled water and resultant turbidity measured with a photo electric cell, using a sodium vapour lamp as a source of light. The turbidity of known samples was compared to the turbidity produced by known standards made up of trichlorobenzene and ethyl alcohol. The method gave a satisfactory range of readings for concentrations varying from 0.30 mg. per liter to 0.03 mg. per liter which, when the original dilutions were taken into account, was sufficient to detect as low as 0.03 mg. per liter of Inerteen in air tested. The method is not specific for the various members

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Sampling at Various Distances from a Source of Inerteen.

The effect of varying the distance at which samples were taken from a closed space over boiling Inerteen was determined with the following results:

<u>Distance Inches</u>	<u>Concentration gr. per liter</u>
14	4.50
20"	1.38
23"	1.14
25"	1.14

Similarly the effect of varying the distance at which samples were taken over an open beaker of boiling Inert was determined with the following results:

<u>Distance Inches</u>	<u>Concentration gr. per liter</u>
5	0.56
10	0.43
15	0.24
20	0.24

The concentrations fall off rapidly and if any cooling surface is above the Inerteen and no air currents are blowing over the surface of the Inerteen, there is much less chance of any appreciable amount escaping into the atmosphere as from a container like a transformer.

Sampling at Various Time Intervals and Temperatures.

Air at 10 liters per minute was passed over a surface of Inerteen (about 10 sq. inches) contained in a flask

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jar at various intervals after the air flow was started and under various conditions of temperature.

	<u>Time of Sampling Hours</u>	<u>Concentration of Trichlorobenzene mg. per liter</u>	<u>Temperature of Trichlorobenzene °C.</u>
At 23°C.	1/2 hour	0.39	23
	2 "	0.51	23
	6 "	0.60	23
At 50°C.	1 "	1.58	50
	4 "	1.79	50
At 90°C.	1 "	1.90	90
Inerteen at 23°C.	6 "	0.58	23

From the foregoing it will be noted that the 6 hour sample with trichlorobenzene at 23°C. gives almost the same concentration (0.60 mg. per liter) as did the six hour sample with Inerteen at 23°C. (0.58 mg. per liter). This suggests the possibility that the bulk of the stuff coming off the Inerteen at that temperature may be trichlorobenzene and that the hazard of Inerteen may thus be the hazards of trichlorobenzene. Fractional distillation of about 335 cc. of Inerteen was carried out in a distilling flask with a 300°C. thermometer reaching to the level of the side arm. The distilling flask was attached to a Liebig condenser and a collecting flask. Samples were collected over temperature ranges from 240°C. to 300°C. (The temperature recorded being that of the vapors around the thermometer bulb. The distance between the bulb and the surface of the liquid was about 6 inches. Although the liquid boiled vigorously several

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ing point and specific gravity was measured for each fraction collected.

Fraction No.	Temperature of Condensing Liquid °C.	Boiling Point Fraction °C.	Spec. Grav.	Vol. %
1	240	208 - 219	1.459	35
2	240-254	228	1.478	45
3	254-280	240	1.493	43
4	280-300	259	1.577	30
Residue	Above 300	-----	1.592	180

Fraction #1 was probably trichlorobenzene (Specific gravity 1.456, boiling point 208 - 219°C.).

Conclusions and Summary:

In investigating the toxicology of Inertean answer to the following questions were sought and are here given as far as possible.

(1). What is the composition of the vapors liberated from Inertean at a maximum working temperature of 90°C.,

Trichlorobenzene only.

(2). What is the maximum concentration of this fraction to which workers might possibly be exposed,

(a) In a closed space 14" over boiling Inertean 4.5 mg. per liter.

(b) In an open space 5" over boiling Inertean 0.56 mg. per liter.

(3). What might be the maximum concentration in room where transformers are placed when the temperature of

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1.8 mg. per liter.

(4). What is a safe concentration as shown by animal experiments. Considering the hazard is due, under ordinary working conditions to trichlorobenzene alone,

(a). From trichlorobenzene data previously obtained, .033 mg. per liter.

(b). From Inerteen data - .048 mg. per liter.

The last figure has been verified by exposing rats for 8 hours per day to such concentrations over a period of weeks.

(5). Is there a simple test that will detect concentrations of Inerteen below .048 mg. per liter,

Yes. If 10 liters of air are drawn at a rate of about one liter every 2 minutes through 1 cc. of ethyl alcohol (cooled in dry ice or an ice salt mixture) and the alcohol poured over the surface of a few cc. of distilled water in a test tube a ring of turbidity will be produced if the concentration is over 0.03 mg. per liter. If a ring is produced, the air tested should be regarded with suspicion and increased ventilation provided.

(6). What is the minimum fatal dose orally? 3 to 3.5 cc. per kilogram.

(7). Is there any skin hazard? Constant exposure

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may render persons exposed susceptible to those dermatoses
which are prone to arise in excessively dry skins.

seen J. Fleming no
J. H. Felt

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