

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF NEVADA

NEVADA POWER COMPANY,
A NEVADA CORPORATION,
PLAINTIFF,

VS.

CV-S-89-555-LDG-LRL

MONSANTO COMPANY, A FOREIGN
CORPORATION;
GENERAL ELECTRIC COMPANY,
A FOREIGN CORPORATION;
WESTINGHOUSE ELECTRIC
CORPORATION, A FOREIGN
CORPORATION; AND DOES I XXV,
INCLUSIVE,
DEFENDENTS.

EXHIBITS 1 - 18
TO THE DEPOSITION OF ROBERT EMMET KELLY, M.D.
VOLUME I
TAKEN ON FEBRUARY 15, 1994

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AN ACNEFORM DERMATERGOSIS

JACK W. JONES, M.D.

AND

HERBERT S. ALDEN, M.D.

ATLANTA, GA.

It is only within comparatively recent times that the dermatologist has interested himself in dermatoses associated with or caused by the daily work. With the advent of a more clearly defined appraisal of the words eczema and dermatitis and the more general use of the patch test, dermatologists have necessarily inquired more and more deeply into the innumerable chemical contacts that occur in the day by day lives of their patients. As well as being conversant with dermatology, the dermatologist must have a working knowledge of the manufacture and content of the many new and old compounds that are handled and applied by large numbers of persons. Hence, more and more dermatologists are becoming industrially minded, and it is dawning on them that the solution of some of their industrial difficulties may aid them in a clearer conception of the dermatoses that are encountered in their every-day practice.

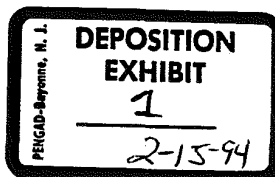
Dermatargoses of the acneform type occurring in persons working with chemicals have been repeatedly reported in Europe and discussed as industrial "chlor-acne."¹ This term "chlor-acne" was first used by Herxheimer² in 1899 to describe an eruption composed of comedones and small sebaceous pustules that occurred on the arms and faces of workers manufacturing chlorine gas electrolytically, using carbon electrodes. It was natural to assume that the chlorine was the causative agent. Bettman³ had observed in March 1897 two patients whose skins were diffusely pigmented, dark, rough and dry, in whom were observed small tenacious comedones associated with numerous small follicular abscesses. Subsequently he reported twenty-one additional cases with similar symptoms in workmen who were engaged in cleaning out an acid tower used in the manufacture of hydrochloric acid. At no time did these men come in contact with free chlorine, but numerous loose

Read at the Fifty-Eighth Annual Meeting of the American Dermatological Association, Inc., White Sulphur Springs, W. Va., May 2, 1935.

1. White, Prosser: The Dermatargoses, ed. 4, New York, Paul B. Hoeber, Inc., 1934.

2. Herxheimer: München. med. Wchnschr. 46:278, 1899.

3. Bettman: Deutsche med. Wchnschr. 27:437, 1901.



derivatives of coal tar were present. Herxheimer later absolved the chlorine but considered the trouble to be due to various chlorobenzenes.

From time to time other reports of the occurrence of "chlor-acne" have been made in the European journals, each author continuing the error of Herxheimer in assuming that the cause of the condition was chlorine. Some of these reports have been indefinite as to the chemical conditions under which the men worked, except that they were exposed to chlorine gas. However, some authors have suspected various chlorobenzene derivatives, such as hexa-chlorobenzene, hexa-chlorethylene, para-nitrobenzene, perchloronaphthalene ("perna") and probably others.⁴ Chlorinated naphthalene was first indicted by Wauer⁵ in 1918 as a cause of acneform eruptions of the skin, the condition being called *Perna-krankheit*. His observations were confirmed by Koelsch⁶ and recently by Teleky.⁷ The latter reported an exhaustive study of a large number of cases occurring in workers engaged in the manufacture of perchloronaphthalene, in which the workers were exposed to fumes of the molten mass and also to the sublimated dusts. In approximately one-half of the workers exposed there developed comedones of a particularly heavy and tenacious type with resultant sebaceous abscesses, containing heavy yellow pus and serum. Considerable improvement resulted when the chlorine content of the mass was decreased from 30 per cent to 8 per cent, and Teleky believed that the disease was due directly to the halogen content of the substance. He mentioned the similarity of the disease to *teeracne* (tar acne) but carried the analogy no further. Individual predisposition appeared to alter receptivity.

Some authors have leaned to the view that the acneform eruption is due to direct external contact with the chemicals (notably Bettman, Hallopeau, Jacquet, Fumouze, Teleky and Koelsch⁸). Others (Lehmann, Herxheimer, Jacobi, Roth and Kobert⁹) considered it to be a

4. Occupation and Health: Encyclopedia of Hygiene, Pathology and Social Welfare, International Labour Office, Boston, World Peace Foundation, 1925, brochures 34 and 285.

5. Wauer, cited in Occupation and Health: Encyclopedia of Hygiene, Pathology and Social Welfare, International Labour Office, Boston, World Peace Foundation, 1925, brochure 34.

6. Koelsch, F., in Ullmann, K.; Oppenheim, M., and Rille, J. H.: *Injuries to the Skin*, Leipzig, Leopold Voss, 1926, vol. 2, p. 303.

7. Teleky: *Klin. Wchnschr.* 6:845 (April 30); 897 (May 7) 1927; 7:214 (Jan. 29) 1928.

8. Bettman; Hallopeau; Jacquet; Fumouze; Teleky, and Koelsch, cited in Occupation and Health: Encyclopedia of Hygiene, Pathology and Social Welfare, International Labour Office, Boston, World Peace Foundation, 1925, brochure 285.

9. Lehmann; Herxheimer; Jacobi; Roth, and Kobert, cited in Occupation and Health: Encyclopedia of Hygiene, Pathology and Social Welfare, International Labour Office, Boston, World Peace Foundation, 1925, brochure 285.

dermatitis due to the absorption of chemical compounds by the lungs or gastro-intestinal tract, with elimination by way of the sebaceous glands. The majority of writers on the subject, particularly Prosser White,¹ have seemed to feel that chlorine as such has little to do with the formation of the comedones and cysts, and they have repeatedly referred to tar and products of the distillation of tar as the prime causative factors. Prosser White rebuked authors for using the term "chlor-acne" at all and expressed the belief that the process is one of the manifold cutaneous reactions produced by tar and its derivatives.

Recently we have had the opportunity to study, with the full cooperation of the manufacturers, an outbreak of acneform eruption occurring in a group of workers engaged in the manufacture of chlorinated di-phenyl. This study has brought out some points in the production of this unusual dermatosis that we feel are important in solving its exact cause. The following case is typically illustrative of the disease as it occurred in the men working in this plant.

REPORT OF A CASE

History.—O. D., a Negro aged 26, began work in the distillation of chlorinated di-phenyl in April 1930 and worked regularly until the latter part of the year 1933. About May of 1933, he noticed the appearance of blackheads on his face, neck, arms and legs. These areas itched slightly. In a short time blackheads began to appear on the chest, back and lower part of the abdomen, around the navel and on the scrotum and penis. Many of these blackheads swelled and became infected, discharging thick pus. The areas healed with difficulty and often left scars. The condition seemed to be progressive until November 1933. When seen in December 1933 the patient complained of lassitude, loss of appetite and loss of libido and said that his cutaneous condition seemed to be improving.

Physical Examination.—On examination he seemed in good general health. His complaint of lassitude was not borne out by anything more than the usual temperament of the Negro toward work. On the forehead, extending within the hair line, and on the cheeks, chin, nose and neck were numerous small, very black, tenacious comedones, their distribution best described as being "peppered" within the skin. Many of the comedones surmounted firm shotlike cysts, which in some areas contained viscid yellow pus. The pustular elements were more noticeable on the neck. Similar shotlike comedones and cysts had appeared on the shoulders, midportion of the back and chest, with an occasional large cyst. A peculiar peppering of the skin with tenacious carbon-colored comedones was apparent around the umbilicus and lower portion of the abdomen. The scrotum and penis were involved in a similar process, the former being given more to the formation of cysts. The outer surfaces of the forearms and anterior thighs showed similar but fewer comedones. The whole eruption was acneform but differed from acne particularly in the lack of a seborrheic appearance of the skin and in the peculiarly deep black of the comedones as well as the general peppered distribution in areas not usually involved in acne vulgaris. A general physical examination had revealed nothing of importance.

Treatment.—He was instructed to scrub his skin thoroughly before and after his hours of work, to wear fresh clothing each day during work and to use a lotion made up as follows: zinc sulfate 4 Gm., potassium sulfurata 4 Gm. and distilled water to make 125 cc. On recommendation he later reported for weekly doses of roentgen radiation according to the usual manner of treating acne vulgaris.

Microscopic Examination.—A portion of the skin of the chest was removed for microscopic study (fig. 1). The chief feature of the sections was noted in the hair follicles and sebaceous glands, in which there were cystic dilatation,

Summary of the Symptoms and Treatment in Sixteen Cases of Acneform Eruption

Case	Age	Race	Type of Skin	Type of Eruption	Time of Exposure	Special Treatment
111	22	White	Seborrhelic; previous acne	Diffuse comedones; few cysts	6 months	Inclison; roentgeno-therapy
2B	28	White	Average	Diffuse comedones; few cysts	5 months	None
3S	32	White	Seborrhelic	Diffuse comedones; large cysts and pustules	Throughout	Inclison; drainage
4W	36	White	Average dry	Diffuse comedones; large cysts and abscesses	10 months	None
5F	28	White	Average	Diffuse comedones; few cysts on face and neck	Throughout	None
6S	30	White	Seborrhelic; previous acne	Diffuse comedones; deep abscesses on neck; severe cysts	10 months	Inclison; drainage
7B	20	Negro	Seborrhelic	Erythematous diffuse comedones; few small cysts	8 months	None
8B	19	Negro	Average	Few scattered comedones; occasional cyst	5 months	None
9D*	26	Negro	Seborrhelic	Diffuse comedones; cysts; small abscesses	Throughout	Roentgeno-therapy
10C	37	White	Average	Scattered comedones; occasional abscess	9 months	None
11G	56	White	Dry	Scattered comedones; occasional abscess	Throughout	Roentgeno-therapy
12P	20	White	Seborrhelic	Few comedones; occasional cyst	2 months	None
13H	37	White	Average	Occasional comedone	Throughout	None
14B	23	Negro	Average	Very few comedones	12 months	None
15F	22	White	Seborrhelic	Scattered comedones	Throughout	Roentgeno-therapy
16P	20	Negro	Seborrhelic	Diffuse comedones; few cysts back and face	?	Roentgeno-therapy

* This case is reported in detail.

destruction of the hair, marked thinning and atrophy of the epithelium of the follicles and a heavy plug of keratinized material which partly filled the cystic cavity. In some areas there was a superficial plug at the surface opening; others showed the surface open and the plug deeply situated. There was no purulent exudate. There were a zone of moderately dense connective tissue surrounding the enlarged follicles, slight edema and infiltration by lymphocytes but no leukocytes. Slight edematous changes were noted in the occasional sebaceous glands present, but they were strikingly few. The sweat glands were normal.



Fig. 1.—Section of skin from the chest, showing histologic changes in the formation of an acneform eruption.

Of the twenty-four men working in the manufacture of chlorinated di-phenyl within the period from the late summer of 1932 to October, 1933, twenty-three were reported to have had an acneform eruption on the face and body. Of the twenty-three, sixteen were examined. These men presented eruptions of acneform character similar in type and distribution to that in the case referred to but varying in severity (table). In many patients numerous small sebaceous abscesses developed, particularly around the collora line, which exuded heavy, tenacious pus,



Fig. 2.—Characteristic appearance and location of an acneform eruption.

and the remaining ulcers were indolent, leaving in their wake much scarring. In two of the cases particularly, many large abscesses developed on the neck and back.

Until very recent times di-phenyl and chlorinated di-phenyl were only laboratory curiosities, and their commercial manufacture was unknown. In the experimental stage of manufacture the apparatus was necessarily crude, and experimenters and workers were exposed for long periods to fumes and dusts containing much chlorine, both free and combined. In the early experiments, as well as in the early manufacture in large

quantities, the di-phenyl was made by heating benzene derived from crude coal tar. When heated in a suitable medium benzene (C_6H_6) becomes di-phenyl ($C_{12}H_{10}$). This substance is then chlorinated at various saturation points, by exposing it under suitable conditions to free chlorine gas. Distillation of the resulting chlorinated di-phenyl¹⁰ results in a purified commercial product (fig. 3). The commercial benzene always has a small quantity of impurities, but some of the cruder commercial benzenes contain relatively large quantities of these impurities, such as xylene, toluene and paraffin. On the heating of the

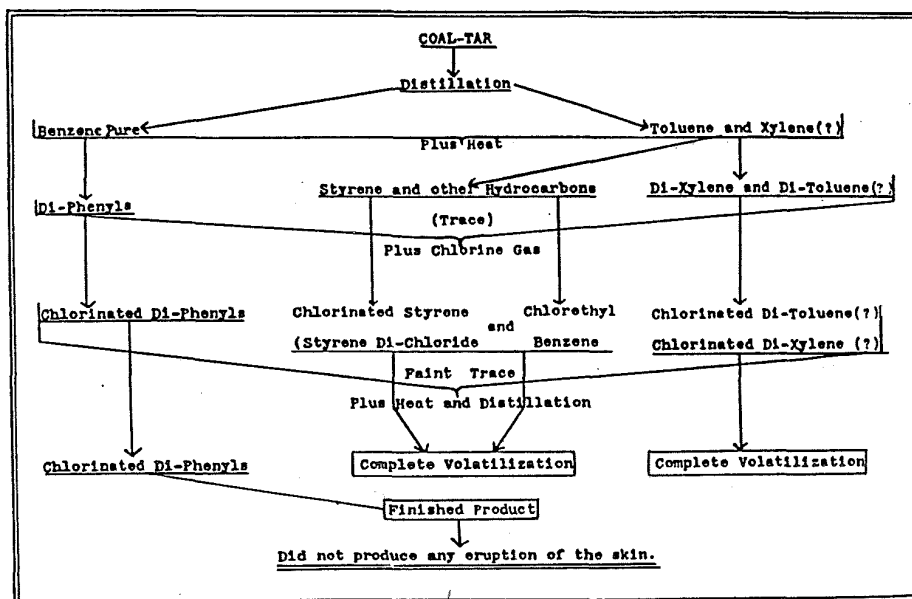


Fig. 3.—Chart illustrating the process of manufacture of chlorinated di-phenyl.

cruder benzenes, the toluenes and xylenes, when present in large quantities, may produce the chemical substance styrene, which has the formula $C_6H_5CH:CH_2$. Styrene when chlorinated (it being inseparable from the di-phenyl) is an unstable compound dropping its chlorine atoms very readily. It is probable that other complex hydrocarbons may be formed from these impurities, which on chlorination become quite as unstable as the styrene. One of these compounds may be chlorethyl benzene ($C_6H_5CH-Cl-CH_3$).

10. Chlorinated di-phenyl consists of a mixture of di-phenyl chlorinated at various saturation points: nona-chloro-di-phenyl ($C_{10}HCl_9$ — C_6Cl_5) etc.

In the experimental stage and in the early manufacture of chlorinated di-phenyl, the men working were exposed for long periods to these chlorinated products. As the demand for the finished product increased, quantitative manufacture was speeded up rapidly, and open stills and heating units were of necessity used until better equipment could be designed and made. But since no physical trouble had developed in the workers previously there was no apparent hazard connected with manufacture. On or about March 1933 the electrical properties of the chlorinated di-phenyl produced in the plant the workmen of which were examined fell below the specifications, and the color of the product deepened. About one month later an acneform eruption was observed on the faces and arms of several of the workers, which became progressively worse, gradually becoming apparent among all the workers engaged in the process at that time. Although one of the men had had a slight acneform eruption on his face in January, little thought was given to it until the eruption appeared in the others. In the summer of 1932, six or eight months previous to the general outbreak of the condition, owing to a difference in price, the crude benzene was purchased from another source, and it was not until the purchase of this particular benzene was discontinued, in October 1933, that the finished product came up to the standard. During the period from March 1933 until October 1933, the eruption continued among the men, and it was present as already described when the men were examined in December of the same year.

The appearance of the eruption coincidentally with the production of a poor grade of chlorinated di-phenyl seemed to indicate a relationship. On due chemical investigation styrene was assumed to be the offending substance causing the low grade of the finished product.

It seems that it may be readily assumed that our problem as to the causative agents of the acneform eruption was connected with the chlorinated products of the impurities present in the crude benzene. However, with the exception of styrene, these products are not known. The problem hence becomes difficult of exact solution, and it is necessary to resort to a reasonable deductive hypothesis.

The exact conditions under which the men contracted their eruption cannot be satisfactorily duplicated experimentally since prolonged mild contact seems to be a deciding factor. Repeated patch tests made on numerous persons with the finished product, chlorinated di-phenyl, and the foreign substances styrene, styrene di-chloride and chlorethylbenzene, have not resulted in an acneform eruption except in one instance. Repeated patch tests made with chlorethylbenzene in the same area produced on the third attempt an erythematous follicular eruption, which persisted only a few days but which was by no means typical.

We could not duplicate this in other instances. Since styrene, chlorinated styrene, chlorethylbenzene, xylene, toluene and their probable products are for the most part lipid solvents, they will produce death and denudation of the epithelium, and one always obtains a chemical burn in response to a patch test made with the styrene and styrene di-chloride. Small quantities mixed loosely with an inert powder or dissolved in ether produce a mild erythematous reaction but no acneform eruption. Patch tests made with the finished product, chlorinated di-phenyl, at no time produced any erythema or evidence of irritation; hence the chlorinated di-phenyl can be absolutely absolved as an irritating agent. In looking over the consecutive history of the development of the eruption, we found it apparent that the men were in some degree exposed to the chlorinated impurities in the benzene for some time before the eruption actually appeared, and that to duplicate the conditions we should have to make repeated patch tests daily for long periods in the same person, a procedure which is not feasible.

As a group, the workers in the dusts and vapors, which were presumably saturated with chlorinated hydrocarbons, were not cleanly, making little effort to bathe after work, and sweating and rubbing enhanced the liability to deposition of the substances on the skin. Theoretically, the chlorinated hydrocarbons, presumably styrene di-chloride and chlorethylbenzene, deposited on the skin gather around the hair follicles and sebaceous glands and are gradually rubbed in. Being lipid solvents, they dissolve sebum and in the reaction, which is modified by the heat of the body and the water present, give off hydrochloric acid, which causes death or irritation of the cells. This in turn causes an excess of cell growth, inflammation, sebaceous plugging and an acneform eruption (fig. 4). This process repeated over a long period may result in severe sebaceous infection, sebaceous abscess and scarring. One would naturally expect this process to occur in the seborrheic areas as well as where clothing might rub the particles into the skin. Also one would expect the process to enhance an already existing acne vulgaris. This occurred in cases 1 and 6 (table 1), the cases in which there was the worst involvement with sebaceous abscesses.

In the beginning an attempt at prevention of the condition was made by being especially careful that all men engaged in the manufacture of chlorinated di-phenyl should have a thorough bath after working hours and that they should wear freshly laundered clothing before starting work. They were also instructed to apply night and morning veterinary white lotion to the affected parts. Some of the patients having the most numerous cysts and follicular infections were instructed to report for roentgen treatment administered in the manner usually employed in treating acne vulgaris. Following the

change in the type of benzene used and the employment of enclosed distilling apparatus and ventilation fans, there was noted a gradual improvement in the acneform eruption. Those patients receiving roentgen radiation improved much more rapidly, but it cannot be said that this form of treatment gave unusual results. The final treatment, of course, was removal of the patient from the offending chlorinated hydrocarbons, and then cleanliness and relief from any infection. All the men showing evidence of cutaneous disease, so far as is known, are greatly improved, or the condition has entirely cleared except for an occasional comedo and a few scars.

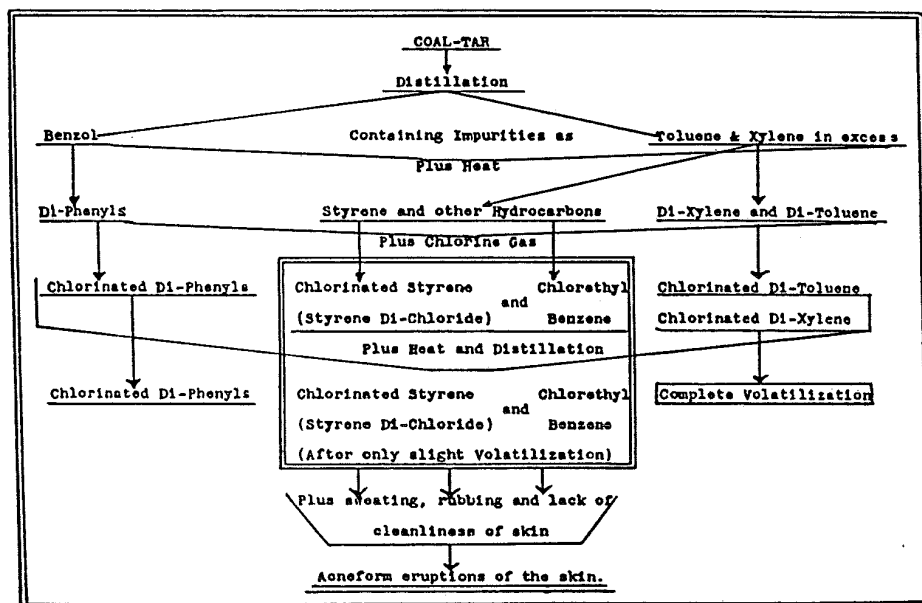


Fig. 4.—Process of manufacture of chlorinated di-phenyl containing impurities which cause an acneform eruption.

When one recalls the cases reported by the European investigators, it is altogether plausible that the chemical process outlined here occurred in most instances. The slow liberation of chlorine in the presence of a lipid solvent (usually chlorinated hydrocarbons) was present in most cases. It is doubtful whether the disease is influenced by the quantity of chlorine present; it is apparently more due to the formation of unstable chlorinated hydrocarbons as well as to the liability of the workers to close contact without a thorough cleansing of the skin and a change from saturated clothing. It is also probable that the disease has little to do with tar itself but more with the products formed by the

chlorination of tar. Hence, the disease cannot be called "chlor-acne" or "tar acne." That it is not caused by internal absorption is evident when one considers the following facts: First, acute intoxications by chlorine rarely occur, since suffocation and death by pulmonary edema ensue even in the presence of relatively small quantities of chlorine gas in the air. Second, when ingested, chlorine and the chlorinated hydrocarbons are so rapidly oxidized and chlorine so readily combines with sodium that opportunity for acne-like eruptions, such as occur in bromine intoxication, is not possible.

In retrospect, we are intrigued by the hypothesis that these theoretical considerations may have some relation to acne vulgaris. The past quarter of a century has seen an enormous increase in the use of coal for heating homes as well as in industry. The smoke stacks of the cities have belched an ever increasing stream of coal smoke—smoke heavily laden with carbonaceous products containing numerous hydrocarbons. It is well known that coal smoke contains hydrochloric acid. Is it not possible that at least some of the acne vulgaris may have a part of its origin in the constant contacts with the chlorinated hydrocarbons of coal smoke?

SUMMARY

We have recorded herein observations concerning an industrial dermatosis of an acneform type. The type of eruption and the causative factors are analogous to those in the previously reported cases spoken of in the European literature as instances of "chlor-acne," tar acne or *Pernakrankheit*. Although exact duplication of the conditions under which the workers acquired the dermatosis cannot be accomplished by experimental means, reasonable deductions as to the cause of the difficulty can be made. The eruption occurred during the manufacture of chlorinated di-phenyl from benzene. The dermatosis occurred at a time when the benzene used contained excessive quantities of toluene, xylene and paraffin as impurities. The heating and chlorination of these impurities probably resulted in the production of styrene di-chloride and chlorethylbenzene, which, we believe, on contact with the skin produced the acneform eruption by the slow liberation of hydrochloric acid and not by internal absorption of any chemical. While many chlorinated hydrocarbons, as brought out by Prosser White,¹ are lipid substances producing death or irritation of the cells by the liberation of chlorine, this is not true in all instances, since the finished product, chlorinated di-phenyl, did not, either experimentally or actually, produce any cutaneous or sebaceous irritation. Irritation apparently depends on the ease with which chlorine atoms are released from the hydrocarbon base. We believe that these dermatoses should be labeled acneform dermatoses resulting from certain unstable chlorinated

hydrocarbons and should not be spoken of as "chlor-acne" or tar acne. The suggestion has been made that some of the acne vulgaris of the skin may be the result of the same type of irritation from soot or coal smoke.

ABSTRACT OF DISCUSSION

DR. OLIVER S. ORMSBY, Chicago: I should like to add an experience I had three months ago that is of interest in this connection. I think that Dr. Jones' paper is extraordinarily interesting and valuable, and the small group of cases I am going to report may add to his troubles rather than solve any of the problems.

About three months ago in Michigan, I saw a patient, through the courtesy of Dr. Milton G. Butler, who presented an extraordinary acneform eruption. This patient was 18 years old and had been observed by Dr. Butler. The eruption in these various cases consisted of acneform pustules and comedones of variable size and depth, with scars, some of which were keloidal. The eruption occurred on the face and neck, particularly over the posterior portion, and extended well up into the scalp. The trunk, arms, thighs and legs were also involved. Two of the patients presented on the back of the neck deep perifollicular pustular lesions, with keloidal scars, such as are seen in Kaposi's dermatitis papillaris capillitii. Both deep and superficial atrophic scars, such as are seen in deep lesions leaving acne, were present in other portions of the neck, face and trunk. The comedones were prominent in all areas, including the legs. They were of variable size, and many were large and deep. The age of the affected patients varied from 18 to 50 years, the younger ones being more severely affected. The condition was apparently produced by contact with an ingredient in a fungicide produced by a chemical company for destroying fungi in lumber. The particular chemical in this fungicide that apparently was responsible for the eruption was sodium tetrachlor-ortho-phenylphenate, which is a yellow powder. Only those who came in contact with it had the eruption. Those who handled the other ingredients of the fungicide and did not come in contact with this particular chemical had no difficulty. As there were apparently no chlorine, bromine or tar radicals in the formula, it is difficult to determine the active factor in the production of the lesions and also whether the eruption was caused by local action by contact or through absorption and internal effects. The eruption was not concerned in any way with acne, and no patient in whom the eruption developed had had acne previously.

DR. MARION B. SULZBERGER, New York: I think that Dr. Jones has contributed a study that will be very interesting when continued. I had an opportunity of seeing a group of patients with acne of external origin about a year and a half ago. The patients were all employed in a factory making radio condensers. Pitch tar and a type of wax (halowax, a chlorinated naphthalene) were used in these condensers. The acne which developed was like that described by Dr. Jones and also included comedones on the legs, as did that which Dr. Ormsby has mentioned. I saw six patients from this one small factory. They were all in the age of puberty or the early twenties, and the group included persons of both sexes.

I believe the most important point in this study is the possible connection between acneform dermatoses distinctly due to external irritants and true acne vulgaris. It is interesting that many derivatives of tar are closely allied to the estrogenic hormone, that certain components of tar are both estrogenic and acnegenic and that the estrogenic hormone must perhaps be incriminated in the production of acne vulgaris. There can be no doubt that acne is often due to stimulation or

irritation of the pilosebaceous apparatus and that hormonal as well as external influences can produce this stimulation. In the patient suffering from acne, who has, of course, come in contact with the estrogenic hormone during intra-uterine life and then has had no further contact with this hormone (or the closely related testicular hormone) until he or she begins to form his or her own hormones, one can readily conceive the pathogenesis of acne vulgaris as being based on an excessive stimulation of the hair follicles, a stimulation produced by the sudden new formation of the hormones at puberty. It is as though the follicles, after being sensitized to the hormones during fetal contact and then passing through a period of freedom from contact, react excessively to the new and massive contacts with the products of the gonads at puberty. I have discussed this subject in great detail elsewhere.

DR. H. G. IRVINE, Minneapolis: I wish to comment on one point and to compliment Dr. Jones on an excellent piece of work and his presentation of it. There was a difference between his situation and that which Dr. Tunnaclyff and I encountered (ARCH. DERMAT. & SYPH. 33:306 [Feb] 1936). In his case the manufacturers called on the medical profession to help solve the problem. Our study was undertaken where lay people were in charge. It shows clearly the need of medical advice in the production of various chemical preparations.

DR. JACK W. JONES, Atlanta, Ga.: I am very grateful for the discussion. I did not have time to go into the various preventive measures attempted in controlling the outbreak or the treatment. In most of the cases the condition has finally cleared up.

Regarding Dr. Sulzberger's discussion, we studied this outbreak and the literature rather carefully for a year and a half or two years, and we are convinced that the etiology in this particular group can be ascribed to external irritation and not to any internal complications. Our idea is that the unstable chlorinated hydrocarbons give off chlorine very easily, whereas the chlorine that is fixed in the benzene ring is comparatively stable. When these unstable chlorinated hydrocarbons come into contact with the skin and enter the hair follicles, they are broken down slowly, giving off chlorine to form hydrochloric acid. We feel that this is the primary cause of the trouble.

SYMPOSIUM
ON CERTAIN CHLORINATED HYDROCARBONS

HARVARD SCHOOL OF PUBLIC HEALTH, BOSTON, MASS.

JUNE 30, 1937

Program

1. The problem of possible systemic effects from certain chlorinated hydrocarbons.

Cecil K. Drinker, MADELEINE FIELD WARREN AND GRANVILLE A. BENNETT

*Department of Physiology, Harvard School of Public Health and Department of Pathology,
Harvard Medical School, Boston, Mass.*

2. Demonstration of laboratory methods for ventilating operations employing chlorinated hydrocarbons. A discussion of permissible concentrations of such compounds in the air.

C. P. YAGLOU, M.M.E.

Assistant Professor of Industrial Hygiene, Harvard School of Public Health

AND

PHILIP DRINKER, Ch.E.

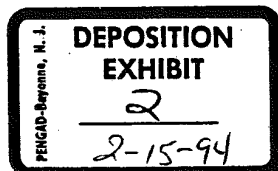
Professor of Industrial Hygiene, Harvard School of Public Health

3. A description of the lesions found in the liver of animals subjected to various chlorinated hydrocarbons.

GRANVILLE A. BENNETT, M.D.

Associate in Pathology, Harvard Medical School

(The second and third papers will appear elsewhere. Copies of them will be sent to all who attended the symposium.)



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THE PROBLEM OF POSSIBLE SYSTEMIC EFFECTS FROM CERTAIN CHLORINATED HYDROCARBONS*

CECIL K. DRINKER, MADELEINE FIELD WARREN AND GRANVILLE A.
BENNETT

*Department of Physiology, Harvard School of Public Health and Department of Pathology,
Harvard Medical School, Boston, Mass.*

THE use of chlorinated naphthalenes and compounds of allied pharmacological possibilities is extremely wide, and with the steady growth of the use of electricity is certain to expand much farther. For years it has been known that many of these compounds cause a troublesome acne, and there is a large literature upon this phase of the subject. Our investigations have not been concerned with chloracne but with the possibility of systemic effects following ingestion or inhalation of such products. In the spring of 1936, the Halowax Corporation, a division of the Bakelite Corporation, called our attention to three fatal cases of jaundice in workmen using chlorinated naphthalenes and chlorinated diphenyl, and requested that the subject be investigated as

rapidly and thoroughly as possible.† In brief these cases were as follows:

Patient 1. Male, age 21. The previous medical history of this man was in no way significant except for the fact that he had an attack of jaundice about 6 weeks prior to his fatal illness. Late in December, 1936, he became badly constipated and had much abdominal pain and distention. When admitted to the hospital he was slightly jaundiced and was evidently very ill. He was somewhat anemic and his skin, particularly upon the arms, face, chest and back, showed many pustules. He died after a brief period in the hospital, and at autopsy was found to

† The Halowax Company makes many products besides chlorinated naphthalenes, and it has come to our knowledge that all of these products are indiscriminately called "halowaxes" by purchasers and users, and are lumped together as possible causes of acne and even of systemic disease. Since "halowax" is merely a trade designation, care should be taken to describe compounds by their chemical names and thus avoid condemnations which are both troublesome and misleading.

* Received for publication June 30, 1937.

have a cirrhosis of the liver with acute yellow atrophy superimposed upon it. This man had been exposed to low concentrations of vapors arising from a mixture of tetra and pentachloronaphthalenes, together with approximately 10 per cent of a refined chlorinated diphenyl. While both he and others engaged in the same work had chloracne, there were no other disturbances of health in fellow workmen, nor was there any precipitating cause for the acute yellow atrophy such as treatment with arsphenamine or exposure to dangerous concentrations of carbon tetrachloride.

Patient 2. This was a young man who died in February, 1936, after an acute illness characterized by jaundice. He had been exposed to fumes arising from a mixture of penta and hexachloronaphthalenes. There is no record of chloracne. The patient worked with a large number of other people of whom but one (Patient 3), a close friend, had significant illness.

Patient 3. Another young man employed with Patient 2. He became jaundiced in March, 1936, and died after an illness of 2 weeks. A careful autopsy resulted in a diagnosis of acute yellow atrophy of the liver. Here again no history could be obtained as to a precipitating cause, and there was no record of preceding attacks of jaundice.

In addition to these three very recent fatalities, we have learned of four other possible cases, none of them fatal. All of these have had jaundice and the entire group consists of isolated individuals who have been picked out of large groups having the same exposure. In but one instance, Patient 1, is there record of antecedent disturbance of health, and the general health of fellow workers has been good.

Such cases have not been reported in the medical literature and only occasionally can one find reference to systemic effects of any sort. For example, Courtois-Suffit (1934) reports on work done by Touraine and his associates (1934) who examined 60 workers

who had been exposed to trichloronaphthalene. Of these 13 were found to have mild digestive complaints, anorexia, nausea and vertigo, but Courtois-Suffit remarks finally, "Absorption is certainly possible and we have for proof of it some of the digestive and general complaints which have been due to it. But they appear to be of little consequence considering the mildness of the digestive troubles and the absence of respiratory phenomena."

In Touraine's cases the exposure was to a trichloronaphthalene, whereas the American cases of acute yellow atrophy were exposed to compounds of higher chlorination. Our own experiments indicate that trichloronaphthalenes require enormous dosage, far beyond anything encountered in industry, in order to produce liver damage. Tel'eky (1927) reported a number of cases of chloracne in persons exposed to chlorinated naphthalenes with a chlorine content ranging from 14 to 53 per cent. He found that the lower the chlorine content the less the acne. Mittelstädt (1935) examined a number of cases of chloracne due to trichloronaphthalene and reported a number of vague general complaints but nothing in the nature of serious disease. Regarding his animal experimentation, Lehmann (1919) reported that animals fed chlorinated naphthalenes refused to eat after a time and that, whether poisoned by inhalation or by feeding, at death showed "peculiar" lesions in the liver. Flinn and Jarvik (1936) gave subcutaneous injections of enormous doses of chlorinated naphthalenes dissolved in paraffin oil to rabbits. The compounds used were as follows:

1. A mixture of tri and tetrachlor-naphthalene.
2. A mixture of tetra and pentachloronaphthalene.
3. A mixture of penta and hexachloronaphthalene.

In addition, sublimates from (2) and (3) were collected in oil and injected subcutaneously.

None of the animals receiving (1) or the sublimate from (2) died, and even after 2 months were quite normal when autopsied. The first death in the animals receiving (3) occurred on the 12th day and the last died on the 26th day. Those receiving the sublimate from (3) were even more severely affected. Autopsy in these animals revealed striking changes in the liver, not, as described, entirely characteristic of acute yellow atrophy but sufficiently suggestive to cause the authors to conclude that "certain chlorinated naphthalenes or impurities contained in them are capable of producing yellow atrophy of the liver in the rabbit."

At the beginning of their paper, Flinn and Jarvik (1936) mention the fact that there have been three cases of acute yellow atrophy of the liver in men working with chlorinated naphthalenes but give no details in regard to them. These cases are undoubtedly the same as those described in the beginning of this paper.

One may summarize the meagre literature upon systemic effects from these substances as follows:

1. With the exception of the mention of acute yellow atrophy by Flinn and Jarvik (1936) there are no reports or even suggestions of serious effects upon human beings.

2. There is evidence (Telcky, 1927) that the degree of chlorination is sig-

nificant in relation to the production of acne. In the work of Flinn and Jarvik (1936) the compounds producing serious liver injury were the most highly chlorinated of those tested, though the chlorine contents as given by analysis vary surprisingly little.

3. There are no published figures upon the amounts of various chlorinated naphthalenes in the air which will produce injury of any sort, and while the work of Lehmann (1919) and of Flinn and Jarvik (1936) point to the liver as a possible site of injury this indication rests upon such extreme dosage as to fail to apply directly to human exposure.

EXPERIMENTAL WORK

In appraising the possible toxicity of any substance met in industry it is first necessary to determine the principal route of absorption. In the case of the compounds under consideration there can be no doubt that inhalation is their chief means of entering the body. They are used not in a great variety of operations and volatilize in varied degree. They are often applied in solution in such volatile solvents as carbon tetrachloride and toluene. The amounts reaching the air under such circumstances are hardly detectable. It will however be shown that carbon tetrachloride adds to the toxicity of the chlorinated naphthalenes and allied compounds, and if there is possibility of inhaling these compounds in other parts of the factory then inhalation of carbon tetrachloride adds a decided hazard. Under such circumstances solvents such as toluene should be used.

Observation in a number of plants causes us to feel that even though

workmen may be extremely dirty and careless, comparatively little of these waxes is eaten. They are tenacious substances, insoluble in water, and if they get on the hands they stick there and are not transferred to the food.

Skin absorption is the third possible means of entering the body. It may occur but at best must be slight when compared to the steady inhalation of finely divided or gaseous material in the air.

They thrive upon a diet very similar to man, and in the case of these chlorinated compounds it is possible that diet may be very significant. Finally, their normal characteristics have been described so well as to make the detection of abnormalities both easy and certain.

Method of exposure.—The inhalation experiments were carried out in four large air-tight wooden boxes, each capable of holding ten rat cages, size

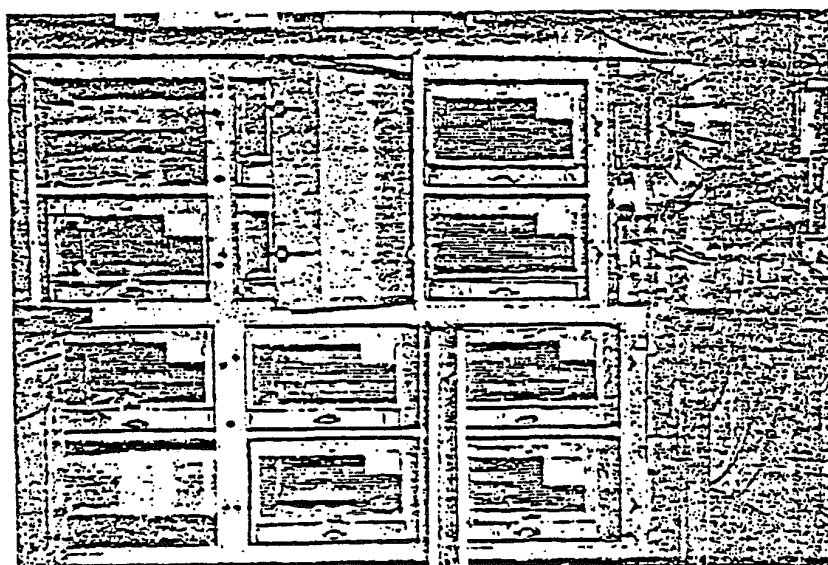


FIG. 1. Front view and inflow end of two boxes with rat cages in place and doors open.

Inhalation experiments are then the most important sources of information, but to them we have added a certain number of observations upon ingestion and subcutaneous injection of various compounds.

Inhalation Experiments

Animals.—White rats have been employed throughout. They permit the use of a large number of animals in a relatively small inhalation installa-

tion. They thrive upon a diet very similar to man, and in the case of these chlorinated compounds it is possible that diet may be very significant. Finally, their normal characteristics have been described so well as to make the detection of abnormalities both easy and certain.

At the center of one end of each box—the inflow end—air was introduced through a pipe 7 inches in diameter (figure 2). Each box was equipped with an individual variable-speed electric blower which blew the air through several feet of 7-inch pipe before enter-

ing the end of the box. An orifice meter was placed in the pipe line, and the flow of air in cubic feet per minute could be adjusted and read off directly from a calibration curve. A vane deflector placed at the entrance of the pipe into the box was adjusted to assure a uniform distribution of the stream of air to the two tiers of cages.

At the opposite end of the box—the outflow end—the air from each box was exhausted through a 7-inch pipe fitted with a damper and connected to a large central exhaust fan (figure 3).

About 4 inches from the entrance of the 7-inch pipe into the box, the fumes of the substances listed were introduced into the inflowing air. Specially designed pyrex glass flasks (figure 4), 7½ inches long and with a diameter of 1½ inches, were used to hold the heated waxes. These flasks were made with a side arm and tube that extended to the bottom. The top of the flask was ground, and into this fitted a short tube 1½ inches in length. This short tube was inserted into a large rubber stopper that fitted tightly into a hole cut out of the 7-inch pipe on the under side (figure 2). The flask in turn was placed in an electric heater made to cover it completely below the side arm and ground glass top. Rubber tubing connected the side arm with a compressed air reservoir and a gentle stream of air blown through the melted wax kept it in motion and assured uniform heating. Into each flask was inserted a long stem-centigrade thermometer which was kept in place and could be read at any time above the 7-inch pipe through which it passed (figure 2).

Approximately 30 gm. of pulverized chlorinated naphthalenes or 20 gm. of

chlorinated diphenyl were placed in the bottom of each flask and melted in the electric heater. Fresh samples were used every other day, but it was often found necessary to add 20 gm. of new material even after one run since so much had sublimed. Whatever the case, the collected sublimate was

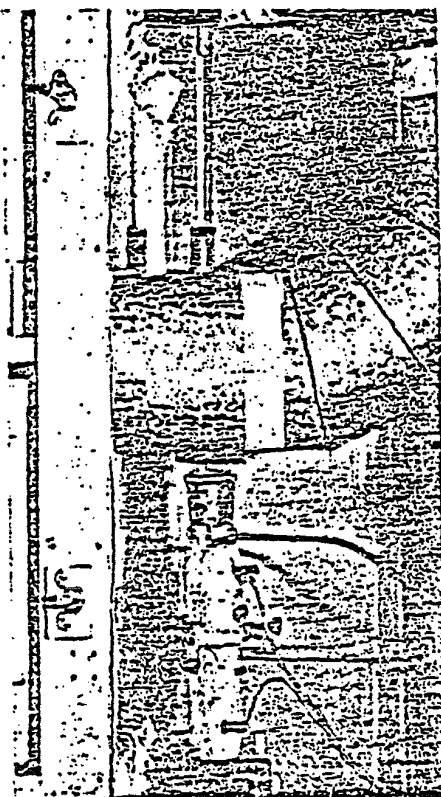


FIG. 2. Electric heater for maintaining chlorinated compounds at a constant temperature in place at inflow end of box.

always removed from the upper part of the flask and a clean top used each day. No sample was ever used for more than two runs.

The flask plus the contents was carefully weighed at the beginning of the run and at the end, and the loss in weight used to calculate the average

amount in a cubic meter of air per minute as determined by a series of

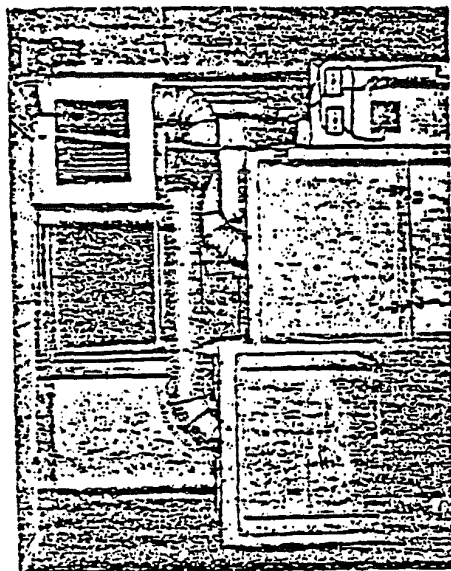


FIG. 3. Outflow end of two boxes showing connections to exhaust system.

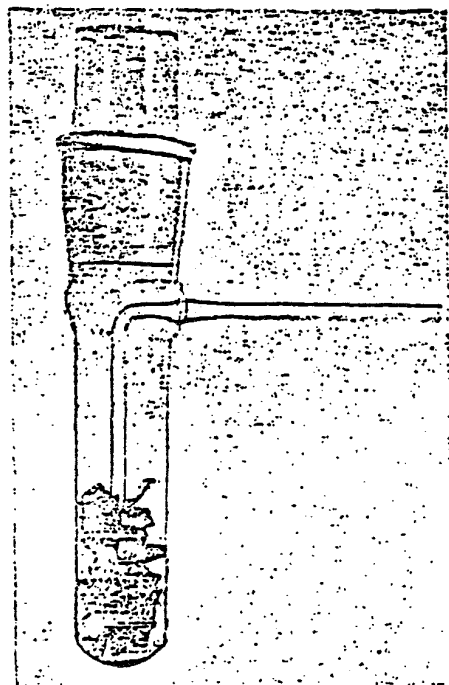


FIG. 4. Pyrex glass flask

flowmeter readings. The figures obtained were not absolute because of slight variations in the air flowing through the boxes and because of deposition of material on the thermometers and on the inside surfaces of the box, but they checked well with direct determinations through air samples.

Approximately 1 hour was allowed for the wax in the flasks to melt and come to a constant temperature. At that time the box doors were tightly closed, air bubbled through the flasks, and the blowers turned on. This was the beginning of the exposure period. By means of rheostats on the fans and dampers in the outflow pipes, the amount of air flowing through the boxes was adjusted and an attempt was made to keep the four boxes as uniform as possible—usually between 165 and 175 c.f.m.

In the first group of experiments the following substances were tested:

1. A mixture of trichloronaphthalenes plus a trace of tetrachloronaphthalene. Chlorine content 49.9 per cent.
2. A mixture of penta and hexachloronaphthalenes. Chlorine content 62.6 per cent.
3. A mixture of 90 per cent penta and hexachloronaphthalenes plus 10 per cent refined chlorinated diphenyl. Chlorine content 63.0 per cent.
4. Chlorinated diphenyl. Chlorine content 65.0 per cent.

The compounds were selected as representing a certain range in chlorination and also because of their industrial importance. In each instance 80 animals were exposed, 10 rats being placed in each cage. They were fed Purina

Dog Chow supplemented by lettuce, eggs, milk and cod liver oil.

This first group of experiments was begun on July 1, 1936 and the last exposure to number 2 (penta and hexachloronaphthalenes) was on November 16th. The exposure to the other three compounds ceased upon November 18th. On October 15th representative animals were taken out of exposure from groups 1, 2, and 4 and were killed after 2 months on December 15th, in order to see whether this clear

wax concentrations were somewhat higher at the inflow end of the boxes. Preliminary runs showed that once properly adjusted, wax concentrations in the air remained very uniform from day to day, but to insure absolute safety readings of temperature, air-flow, etc. were made every night between 10:00 and 12:00 p.m. as well as on starting and stopping. At different times during the course of the experiment tests for free chloride were made but were uniformly negative and

TABLE 1

CONDITIONS MAINTAINED IN INHALATION EXPERIMENT FROM JULY 1 TO NOVEMBER 16 AND 18

MATERIALS	CHLORIDE CONTENT	TEMP.	AV. CONCENTRATION OF AIR IN BOX		TOTAL EXPOSURE	AV. DAILY EXPOSURE
			mg./cu. ft.	range	hours	hours
Trichloronaphthalenes plus traces of tetrachloronaphthalene.....	49.9	150-160	1.31	High 2.60 Low 0.10	1896	16
Penta and hexachloronaphthalenes....	62.6	160-170	1.16	High 2.19 Low 0.51	1864	16
90% penta and hexachloronaphthalenes plus 10% chlorinated diphenyl.	63.0	165-175	1.37	High 3.17 Low 0.64	1896	16
Chlorinated diphenyl.....	65.0	165-175	0.57	High 1.19 Low 0.22	1896	16

period would bring about recovery in the affected livers. Animals from group 3 were similarly removed from exposure on October 4th and killed for examination on December 4th.

The average length of exposure was 16 hours daily for 6 days a week. Each morning at about 9:00 exposure ceased, and between this time and 4:00 p.m. the rats were cleaned, fed, weighed, etc. In order to secure uniformity of exposure the cages were shifted on a regular schedule, since

showed that under the temperatures used no decomposition occurred.

Table 1 shows the temperatures at which the various substances were held, the average concentrations per cubic meter of air, and the average exposures from July 1 to November 16 and 18.

The concentration of chlorinated naphthalenes and chlorinated diphenyl in the air of workrooms.—Table 1 shows that animals have been exposed to varied concentrations of the substances under

test. The concentrations employed may be regarded as fairly representative of industrial experience. Prior to the initiation of inhalation experiments a number of estimates of chlorinated hydrocarbons in the air of different factories were made and the

in question is passed over heated platinum in an electrically heated quartz tube and the effluent gas scrubbed in a column of glass beads moistened with sodium carbonate containing a trace of sodium sulfite. The beads are then washed down and the chloride deter-

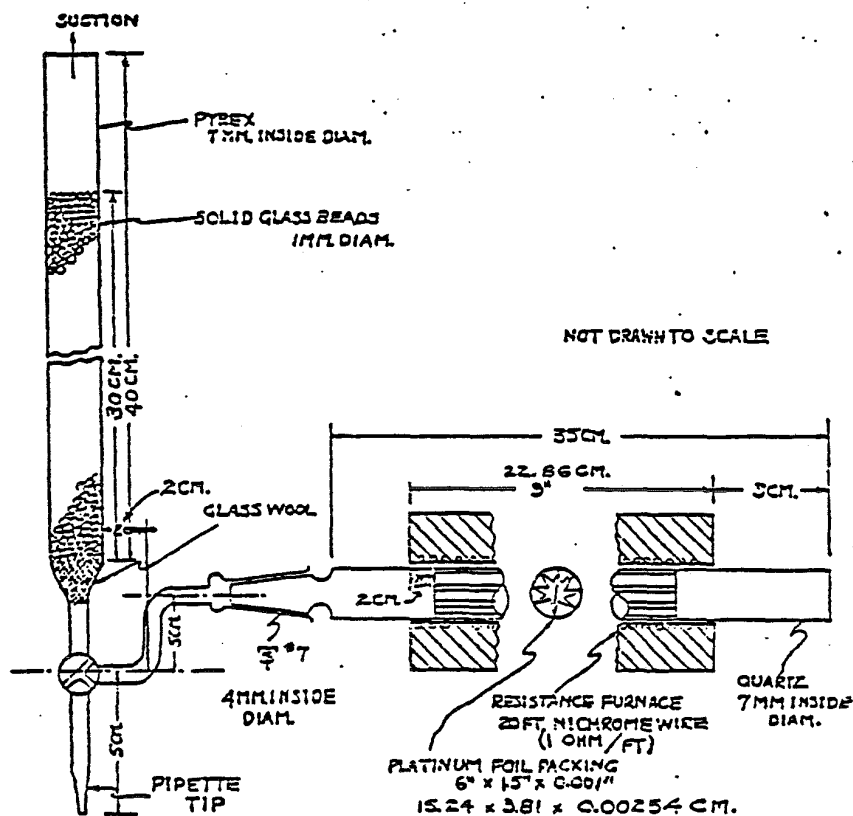


FIG. 5. Dimensioned sketch of combustion tube and absorption apparatus.

concentrations chosen for inhalation experiments depended on these examinations. The technic of analysis and the apparatus employed were the work of Frederick R. Millhiser and William F. Hemperly.

The method and apparatus used for determining concentrations of chlorinated hydrocarbons in air were adapted from well-known procedures. The air

mined nephelometrically as silver chloride.

Tebbens (1937) has recently described a method and apparatus which should be equally satisfactory for this work. In figure 5 we show a dimensioned sketch of the combustion tube and absorption apparatus as used by us in both our laboratory and field problems. The absorption tube is

somewhat easier to wash down than is Tebbens's but the essentials of the two devices are the same.*

In both cases conversion of the chlorinated hydrocarbons to hydrochloric acid and subsequent absorption as sodium carbonate should be complete. In our case concentrations in air are apt to be very low—the objective is less than 1 mgm. per cu. m. Consequently the amount of silver chloride actually formed in the final reaction is so small that it can not be determined either gravimetrically or by titration. For this reason we have been forced to use the nephelometric procedure which is sensitive to concentrations as low as 0.1 mgm. per cu. m. of air.

It is doubtful if the sampling rate should exceed 1 liter per minute as conversion of the chlorinated compound to hydrochloric acid and its subsequent absorption are not efficient unless the velocity of the gas stream is low. Another precaution to be noted is that the method is not selective—any chlorinated substance will be determined. Furthermore it does not distinguish between solid particles and gases but determines them all alike and the results must, of course, be computed in terms of total chlorine.

At the present time we possess information as to the amounts of chlorinated naphthalenes in the air of 30 different plants, and in a number of instances the measurements have been repeated several times. Frequently the amounts have been greater than those used in our experiments, but it must be remembered that the rats

have been exposed for 16 hours to an atmosphere constantly impregnated with the substance under test, whereas human exposure is usually a variable quantity, intense for a short time and then negligible. It is our opinion at the present time that the concentrations of chlorinated hydrocarbons used in our experiments would be dangerous for workers in the case of compounds above trichloronaphthalene in chlorination. Fortunately it is easy to ventilate processes of manufacture which require these substances and to reduce air contamination practically to the vanishing point. Such treatment of the problem at once removes both the possibility of systemic poisoning and the annoyances that arise from cases of acne.

Results of Inhalation Experiments.

1. Animals exposed to a mixture of trichloronaphthalenes plus small amounts of tetrachloronaphthalene. Living animals were apparently entirely normal. Autopsies performed near the end of exposure seemed to show slight swelling of the liver, and microscopic examination occasionally showed swollen and hypergranular liver cells. The changes were, however, never more than slight.

2. Animals exposed to a mixture of penta and hexachloronaphthalenes. No abnormalities were observed in the living animals. Rats were killed and autopsied every 6 weeks. In the first animals sacrificed liver changes were observed. These were swelling of cells, slight granulation and hyalinization. In September and October these conditions were somewhat more advanced, and in November the process became stationary. There were highly

* The combustion and absorption apparatus suited to field sampling is now made by Willson Products, Inc., Reading, Pa. The equipment includes flowmeter and suction pump with a carrying case.

granular cells, hyaline inclusions and mitotic figures, but no more than 2 months previously.

3. Animals exposed to a mixture of 90 per cent penta and hexachloronaphthalenes plus 10 per cent refined chlorinated diphenyl. No abnormalities were seen in the living animals. After 6 weeks the livers showed changes similar to those in the animals exposed to penta and hexachloronaphthalenes. These advanced in grade during August and September and then became stationary.

4. Animals exposed to chlorinated diphenyl. No abnormalities were seen in the living rats. After 6 weeks' exposure there was slight liver damage which advanced during the next 2 months. The changes consisted in slight to moderate swelling of the liver cells, an increased granularity and many mitotic figures. Hyalinization was always present as a result of inhalation of chlorinated diphenyl.

Summary of the first inhalation experiment.—In these experiments careful observation of appearance, body weight, activity, blood, and urine showed no abnormalities of any sort. Yet after 6 weeks' exposure all the compounds with chlorination above trichloronaphthalene caused minor degrees of liver damage, and no changes whatsoever in other organs. There was no acute yellow atrophy or anything suggesting it except that a slight degree of liver damage was always present and was quite clear in the liver sections examined microscopically. This damage had no detectable effect on the health of the animals. They held their weight, ate and behaved normally, being in every respect similar to the many people who have been

exposed to these compounds without illness of any sort.

The functional appraisal of the liver damage caused by certain chlorinated naphthalenes and by chlorinated diphenyl.—There are no tests of liver function useful in such minor degrees of liver damage as were produced in these experiments. Indeed the animals resisted the injury so perfectly as to display no abnormalities except upon histological examination of the liver. The situation was perhaps similar to that met in industry, where, barring acne, the health of workers in these compounds has been good with the exception of the fact that in isolated instances jaundice has occurred which upon at least three occasions has gone on to acute yellow atrophy.

During recent years this disease has been seen following administration of carbon tetrachloride, arsphenamine and cinchophen. In the case of carbon tetrachloride it is known that a low calcium diet and alcohol favor the production of liver damage. For the acute yellow atrophy that occasionally complicates use of the other two drugs no cause can be assigned. One cannot produce acute yellow atrophy with arsphenamine, but somehow or other this now and then does happen to patients under antisyphilitic treatment.

It occurred to us that something of the same sort might be involved in this problem. The human cases have been scattered and few. They have been isolated instances out of large groups of healthy employees who have had equal degrees of exposure. It was our idea that perhaps many of these people got liver changes such as existed in our rats, changes not recognizable through

any means other than autopsy examination. If, upon the substratum of such changes, they got an acute liver disturbance, acute catarrhal jaundice, not a very common disease but one which any of us may experience, would this relatively innocuous disease go over to acute yellow atrophy?

Knowing that our inhalation rats had liver changes, but changes too slight to cause recognizable symptoms, we decided to test their resistance to substances known to cause liver destruction. Carbon tetrachloride and alcohol were selected. Having information that 1.0 cc. of carbon tetrachloride plus 1.0 cc. of ethyl alcohol per kilogram would kill 14 per cent of normal white rats, we reduced the dose to 0.75 cc. carbon tetrachloride and 0.75 cc. of ethyl alcohol.

This mixture was given by stomach tube to the following groups of animals and with the results found in the following tabulation.

Rats fed 0.75 cc. per kgm. each of carbon tetrachloride and ethyl alcohol by stomach tube

1. *Trichloronaphthalene plus trace of tetrachloronaphthalene.* Fed at 9:00 a.m., Nov. 10, 1936. 10 rats. No deaths.

2. *Penta and hexachloronaphthalenes.* Fed at 9:30 a.m., Nov. 10, 1936. 10 rats.

3 died Nov. 11th between 4:30 and 10:30 p.m.

1 died Nov. 12th at 3:20 p.m.

1 died Nov. 12th at 4:30 p.m.

1 died Nov. 13th between 9:30 and 11:30 a.m.

1 died Nov. 15th between night of Nov. 14th and 12:30 p.m. Nov. 15th. 2 died Nov. 16th between 10:15 p.m. Nov. 15th and 9:00 a.m. Nov. 16th (stiff).

3. *90 per cent penta and hexachloronaphthalenes and 10 per cent chlorinated diphenyl.* Fed at 10:00 a.m., Nov. 10, 1936. 10 rats.

1 died Nov. 10th between 5:00 and 10:30 p.m.

5 died Nov. 11th between 4:30 and 10:30 p.m.

1 died Nov. 11th between 4:30 p.m. and 9:00 a.m. Nov. 12th.

2 died Nov. 12th about 1:00 a.m.

1 died Nov. 13th between 5:00 and 10:00 p.m.

4. *Chlorinated diphenyl.* Fed at 10:00 a.m., Nov. 10, 1936. 10 rats.

1 killed Nov. 10th at 10:30 p.m. (almost dead).

2 died Nov. 11th between 4:30 and 10:30 p.m.

1 died Nov. 12th before 8:30 a.m. (stiff).

1 died Nov. 13th between 9:30 and 11:00 a.m.

1 died Nov. 16th between 10:15 p.m. Nov. 15th and 9:00 a.m. Nov. 16th (stiff).

Controls: Fed at 9:30 a.m., Nov. 12, 1936. 10 rats. 1 killed Nov. 13th for normal liver.

This tabulation summarizes into the facts that:

1. No normal rats were killed by carbon tetrachloride and ethyl alcohol.

2. No trichloronaphthalene exposed rats were killed, and this finding agrees with our inability to find lesions of moment in the livers of the animals that inhaled this substance.

3. The penta and hexachlorinated naphthalene, the mixture of these with 10 per cent chlorinated diphenyl, and finally the latter substance alone seem to have produced conditions lowering the resistance to an agent capable of producing serious liver disease.

4. The sole lesion produced by this test of liver function was acute yellow atrophy usually accompanied by jaundice.

This test of liver function was accomplished with a substance which itself is an organic chloride and curiously enough it is the only substance we were able to find that was effective.

In animals with the minor degree of liver injury which has been described we were unable to produce acute yellow atrophy or any variety of liver effect with arsphenamine, cincophen, butyl chloride, ethylene chloride and tetrachlorethylene, but carbon tetrachloride was uniformly effective in disclosing the existence of liver damage.

Recovery from liver damage following removal from exposure.—Animals exposed to trichloronaphthalenes plus traces of tetrachloronaphthalene being practically normal on removal from exposure need no consideration. In the case of the penta and hexachlor-

signs of liver injury will be many months in returning to normal.

The effect of high concentrations of trichloronaphthalenes with traces of tetrachloronaphthalene.—The first group of inhalation experiments showed that this material in concentrations averaging 1.31 mgm. per cu. m. of air was relatively innocuous, judged both by direct observation and by the carbon tetrachloride test. This is an interesting fact since such compounds cause acne, though less potently than substances of higher chlorination. In order to explore the matter further one of the inhalation boxes was arranged

TABLE 2
CONDITIONS MAINTAINED DURING INHALATION OF HIGH CONCENTRATIONS OF
TRICHLORONAPHTHALENES PLUS TETRACHLORONAPHTHALENE

MATERIAL	TEMP.	AV. CONCENTRATION OF AIR IN BOX		TOTAL EXPOSURE	AV. DAILY EXPOSURE
		mg./cu. m.	range	hours	hours
Trichloronaphthalenes plus traces of tetrachloronaphthalene	137-200	10.97	High 16.49 Low 5.78	1232	16

naphthalene, rats after 2 months' removal from exposure still showed swollen liver cells, increased granularity, hyalinization and mitotic figures. The condition was not advanced as compared with rats killed at the time of removal from exposure but on the other hand 2 months were insufficient for recovery.

The same findings were true of the mixture of penta and hexachlorinated naphthalenes and chlorinated diphenyl and for chlorinated diphenyl alone.

Apparently the changes induced in the liver cells by these substances are exceedingly persistent and one must expect that an individual showing any

so that fumes from four glass containers were delivered to the air line instead of one. This resulted in the conditions shown in table 2. The animals subjected to these conditions showed no clinical effects of any sort. After 1 month the liver cells were slightly swollen and over-granular and there were occasional mitotic figures. These changes were similar to the early effects of more highly chlorinated compounds, and progressed only slightly during the third and fourth months. When rats in this condition were given carbon tetrachloride and alcohol, in some instances their livers showed massive central necrosis and

in others this did not develop. Fifty rats were used in this experiment and one may conclude that while the trichloronaphthalenes are in no degree as toxic as those of higher chlorination they are not entirely free from effects upon the liver if high enough concentrations are inhaled over long periods.

The effects of high concentrations of penta and hexachloronaphthalenes.—There can be no doubt as to the damage done the liver by these compounds but in the concentrations so far reported no symptoms were caused that could be recognized clinically and nothing approaching acute yellow

the liver after 35 days of freedom from inhalation. It is thus evident that penta and hexachloronaphthalenes are markedly toxic compounds and that recovery from their effects is extremely slow.

The effects of penta and hexachloronaphthalenes, the mixture of these with 10 per cent chlorinated diphenyl, and of chlorinated diphenyl alone, when inhaled in low concentrations through an 8-hour day instead of a 16-hour day as in the first experiment.—Since steady human exposure to any of the compounds tested would invariably be for 8 hours rather than 16-hour periods, a further

TABLE 3

CONDITIONS MAINTAINED DURING INHALATION OF HIGH CONCENTRATIONS OF PENTA AND HEXACHLORONAPHTHALENES

MATERIAL	TEMP. °C.	AV. CONCENTRATION OF AIR IN BOX		TOTAL EXPOSURE hours	AV. DAILY EXPOSURE hours
		mg./cu. m.	range		
Penta and hexachloronaphthalenes.....	137-195	3.38	High 14.0 Low 5.75	608	16

atrophy occurred except through the use of the carbon tetrachloride test. On December 1, 1936, 80 rats were subjected to the conditions summarized in table 3. This experiment was terminated on January 21. All of the animals lost weight and appetite and deaths began after 8 days of exposure. Fifty-five rats died, most of them heavily jaundiced, 8 were killed for microscopic examination, 9 were killed by the carbon tetrachloride test and 8 lived through the period of exposure. The livers of the animals killed for examination showed marked fatty degeneration, central in type with necrosis of liver cells. Surviving animals showed pronounced changes in

inhalation experiment was arranged under the conditions shown in table 4 which are quite comparable to those in table 1 except for the shorter inhalation periods. Eighty rats were used for each of the three compounds tested. None at any time showed the slightest evidence of illness. Microscopic examination beginning after 6 weeks' exposure showed swollen liver cells, excess granulation, hyaline inclusions and occasional mitotic figures. These changes did not progress and were very similar to those in the animals exposed for 16 hours under the conditions set forth in table 1. The carbon tetrachloride test was uniformly fatal to them and one must conclude that con-

centrations of these compounds such as were employed cause a certain degree of liver damage even if inhaled for but 8 hours daily. This injury is resisted successfully by the rats just as was the 16-hour injury but it is none the less there and destroys the ability of the animal to resist the carbon tetrachloride test.

Summary of inhalation experiments. The findings that have been described briefly will be amplified upon the pathological side in a further paper by Dr. G. A. Bennett. What has been given is, however, sufficient to indi-

men never inhale enough of any of these substances to get acute yellow atrophy. They may, however, acquire a substratum of liver damage upon which acute yellow atrophy may develop. Experience in a number of plants has shown how easy it is to reduce concentrations of these compounds practically to the vanishing point, and every effort should be made to attain such conditions.

Gross Feeding Experiments

The various compounds used were ground as finely as possible and mixed with a standard balanced ration for

TABLE 4
CONDITIONS MAINTAINED DURING INHALATION OF LOW CONCENTRATIONS OF THREE COMPOUNDS DURING 8-HOUR INSTEAD OF 16-HOUR PERIODS

MATERIAL	TEMP.	AV. CONCENTRATION OF AIR IN BOX		TOTAL EXPOSURE	AV. DAILY EXPOSURE
		mg./cu. m.	range	hours	hours
Penta and hexachloronaphthalenes.....	149-193	1.44	High 2.53 Low 0.42	920	8
90% penta and hexachloronaphthalenes plus 10% chlorinated diphenyl.....	150-197	1.66	High 3.30 Low 0.56	912	8
Chlorinated diphenyl.....	153-174	0.93	High 3.23 Low 0.03	920	8

cate that compounds more highly chlorinated than trichloronaphthalene are capable of causing liver injury when inhaled steadily in quite low concentrations. It is an extraordinary thing that even the most searching examination fails to show injury in any other region. It is not easy to grade the toxicity of the different compounds tested, but the chlorinated diphenyl is certainly capable of doing harm in very low concentrations and is probably the most dangerous. Industrial experience combined with these experiments make it appear probable that work-

the white rat. The food was placed in a single container, at which the 10 animals in a cage had an equal share. The amount of the compounds added being reduced as the number of rats lessened during the feeding period. The experiments were designed to give an idea as to the possible toxicity of the compounds selected and, if toxicity was observed, some idea as to the site or sites of damage.

The experiments were successful in both respects, and indeed one may anticipate that future appraisals of the possible toxicity of chlorinated hydro-

carbons may often be made by simple feeding and do not require the elaborate apparatus and the expense necessary for inhalation experiments. The essential reason for this opinion resides in the identity of the lesions produced by both methods, and the fact that the different compounds so far studied seem to arrange themselves identically so far as toxicity is concerned whichever way they are administered.

Trichloronaphthalene plus traces of tetrachloronaphthalene.—Feeding began on May 4, 1936 and continued until November 2, 1936. In the beginning 3 gm. of this mixture were added to the food for 10 rats each day, and this concentration was maintained throughout the experiment. All the animals were killed except the last which died on Nov. 2. There was no loss of weight and no appearance of wasting illness of any sort. The single death was due to some variety of respiratory infection which had no relation to the material inhaled.

Histological examination showed slight changes in the liver but nothing of great significance.

Tetra and pentachloronaphthalenes.—Feeding began on June 29, 1936. A dosage of 0.5 gm. daily was employed. All the animals sickened gradually and were either killed or died by August 29, 1936.

At autopsy both grossly and histologically the liver was the single organ affected. The liver cells showed swelling, hypergranulation, hyaline inclusions and vacuolation. Here and there necrotic cells were found. There was a tremendous accumulation of fat.

Penta and hexachloronaphthalenes.—Feeding began May 4, 1936, a 3 gm. dosage being used. By June 6, nine

rats had died. The last one was obviously ill and was killed for autopsy examination. All animals lost weight from the beginning and were ill. At autopsy the liver alone was affected, the lesions observed being similar to those that have been described for tetra and pentachloronaphthalenes but worse.

90 per cent penta and hexachloronaphthalenes and 10 per cent chlorinated diphenyl.—Feeding began May 4, 1936 on a 3 gm. dosage. On May 16th feeding was stopped but all the animals went on to death, the last dying on June 8th. An autopsy the liver lesions were extremely severe and of the usual type.

On account of the high toxicity of the 3 gm. dosage, four rats were given a 0.5 gm. dose every other day. Feeding began June 24, 1936 and the last animal was killed Sept. 11th. There were no deaths but all the animals lost weight. At autopsy the liver as usual was the single organ affected, the lesions being characteristic and extensive.

Chlorinated diphenyl.—Feeding began May 4, 1936 on a 3 gm. dosage and was discontinued on May 10th. Seven of the 10 rats were dead by May 12th. The three remaining rats gained in weight but were sacrificed for autopsy purposes on July 8th. The liver changes began at once. There were no changes in other organs.

A second group of 10 rats was fed a much smaller dose—0.5 gm. every other day. Feeding began May 20, 1936. The first rat died on May 29th and four more before June 24th. The remaining were sacrificed. Those rats that died showed losses in weight, while those sacrificed had recovered

their initial loss in weight and were gaining. The liver lesions were similar to those found in rats fed penta and hexachloronaphthalenes plus 10 per cent chlorinated diphenyl but not so marked.

Summary of gross feeding experiments.—Of the various materials fed rats in large doses trichloronaphthalene plus traces of tetrachloronaphthalene was quite innocuous. Tetra and pentachloronaphthalene showed definite liver damage. Penta and hexachloronaphthalenes caused a similar grade of injury. The addition of chlorinated diphenyl to penta and hexachloronaphthalenes increased the toxicity. Chlorinated diphenyl alone produced liver lesions but in the dosage used was less effective than when mixed with highly chlorinated naphthalenes. In no case did the compounds used produce acute yellow atrophy but the lesions observed indicate this might be possible if one found a dosage which could act for the proper period of time.

Feeding Precise Doses by Stomach Tube

The compounds employed were suspended in gum acacia. In figuring the dosage the total amount a man of 50 kg. would inhale in an 8-hour day assuming an air concentration of 20 mgm. per cu. m. was first calculated and reduced to milligrams per kilogram. The rats and rabbits received this dose each day. The compounds used were those employed in the gross feeding experiments and the results were essentially similar though the lesions were less severe.

Subcutaneous Injections

The same gum acacia suspensions were injected subcutaneously into rats

and rabbits, the dosage being calculated on the basis of 4 mgm. per cu. m. of air. Again similar results were obtained. In all such experiments there must of necessity be differences in the degree of effect but invariably the liver was the sole organ affected and the lesions were those already described many times.

DISCUSSION

These experiments leave no doubt as to the possibility of systemic effects from the chlorinated naphthalenes and chlorinated diphenyl. As in the case of the effects upon the skin, the degree of chlorination seems to determine the systemic toxicity, and it is a striking thing that when trichloronaphthalene is reached systemic effects are never marked and are produced with the greatest difficulty. It is most remarkable, too, that all the compounds tested attack the liver and the liver alone. During the past few months we have determined the organically combined chloride in the livers of animals very severely poisoned by penta and hexachloronaphthalenes but have found no increase over normal figures, though the livers, as determined histologically, were very severely affected. At the present time we are conducting inhalation experiments on a chlorinated diphenyl containing 55 per cent of chlorine instead of 64 per cent as in the case of the experiments reported in this paper and on a compound with a chlorine content between tri and tetrachloronaphthalene. We are also determining the degree to which the diet may increase or decrease toxicity, this being suggested by similar work upon carbon tetrachloride.

In the basis of these experiments

and on many field determinations of different compounds in the air of work-rooms, it appears safe and it is certainly easily attained, to ventilate so that the air breathed does not contain more than 0.5 mgm. per cu. m. of any of these compounds above trichloronaphthalene. In the case of the latter compound concentrations of 10.0 mgm. are permissible. We know from many examinations in many different plants that such concentrations have been greatly exceeded during the past 20 years, and we are conscious of the fact that our rat exposures have been inexorably constant whereas human exposure is never so ordered. Time and careful observation may change these opinions as to standards, but today we are convinced they are safe. Impregnating tanks and other arrangements utilizing the chlorinated hydrocarbons are easy to hood and to safeguard. Compared with benzene, lead tetraethyl and many other com-

pounds, these substances are very little toxic and operations employing them can easily be safeguarded. It may be argued that where possible trichloronaphthalene should be used, but this compound will cause acne and if employed very carelessly might do more. Furthermore, higher chlorination is often essential for highly practical reasons. The solution consists in thoroughly adequate ventilation plus good housekeeping around all wax containers.

A final word of caution bears upon the use of carbon tetrachloride as a wax solvent. Obviously this compound adds readily to the toxicity of the highly chlorinated waxes. If carbon tetrachloride is used, ventilation should be excellent, but in our opinion it would be better to dispense with carbon tetrachloride and depend on other solvents, especially upon those containing no chlorine.

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DISCUSSION

DR. CECIL K. DRINKER: We have had quite a long morning, but since Dr. von Oettingen has to leave, I am going to ask him to say a word.

DR. W. F. VON OETTINGEN (Director, Haskell Laboratory of Industrial Toxicology, Wilmington, Delaware): I wish to congratulate Dr. Drinker and his group on the very interesting and excellent work on the subject. I also wish to congratulate the Halowax Corporation on the fact that they didn't spare any effort to elucidate the problem so that the dangers which apparently are connected with the use of this material can be controlled.

I personally believe that there is no toxic material which cannot be used safely if it is handled properly. In order to handle such materials properly during the different operations it is absolutely essential to be familiar with the toxicity and especially with the mechanism of the action. Since there is no animal which corresponds in all its physiological characteristics to the human being it is important that such compounds be studied with different species of animals so that one can get a cross-section of the potential dangers.

In addition, this fact illustrates the importance of the pre-employment and periodical examination of workers in such work. As was pointed out by Dr. Drinker, there are individuals who are apparently more susceptible to certain toxic agents than others. On the other hand, we have to try to detect the very first signs of incipient poisoning in order to prevent more serious damage. In the present instance it is very difficult to find these very first symptoms because there are no adequate methods, but I trust that in time the scientific profession will supply the medical profession with adequate methods which will allow the physician to determine incipient damage of the organ before serious clinical symptoms become manifest.

Thank you.

Afternoon Session

DR. CECIL K. DRINKER: In this discussion I will call on one or two individuals and I shall expect others to speak as their notes

incline them to. First of all I shall ask Dr. Sayers if he will open the discussion for us.

DR. R. R. SAYERS (Chief, Division of Industrial Hygiene, National Institute of Health, U. S. Public Health Service, Washington, D. C.): Dr. Drinker, Ladies and Gentlemen: It gave me a great deal of pleasure to have the opportunity to listen to the excellent and interesting discussion and presentations made this morning. Dr. Drinker, and I might add, the members of the Halowax Corporation, have discussed this matter with us, as well as the manufacturers of the diphenyls.

We have been doing some work but not nearly as extensive as that carried on here. The work that we have been doing seems to confirm the work that Dr. Drinker has carried on. I think there are certain parts of it that we can very well keep in mind.

We are dealing with toxic substances, but we are dealing with toxic substances each and every day in industry—harmful substances, hazardous substances. That these can be controlled is well known, no matter what they are, and that we can use them and use them in a safe manner. Dr. Drinker has called your attention to the fact that this apparently has been done and can be done in any of the plants where halowax is used. I have information from one or two of the other men who are in the audience that in plants where damage has been done—skin damage and apparently liver damage to some of the workers—the manufacturers have since corrected the conditions and apparently have adequate engineering control supplemented with adequate medical control.

After all, the proof of the pudding is in the eating. If the man does not get the disease your engineering control is successful. If he does get the disease, no matter what you have done in the way of engineering, you haven't been successful. We must go hand in hand. Neither profession alone can be successful. We have got to work together whether we like it or not. I happen to like it.

There is one other thing. As far as materials are concerned I think we ought to be

smart enough to use every one of them successfully. If this material is particularly adapted for a certain purpose we should use it. Another material that was used previous to this for insulation purposes, which was flammable, caused a disaster in which approximately 167 men lost their lives. I say approximately 167 because they were never just sure whether it was 167 or 168. It might have been one or two more but they were quite sure that 167 lost their lives. It happened when they had insulated a cable with this material. They had an armored electric cable. It was covered with lead armor on the outside. It was 1200 feet long and was being lowered into a mine shaft approximately 3000 feet deep. It was lashed to a steel rope. It slipped and fell to the bottom of the shaft. When the foreman was inspecting it he set it on fire with his carbide cap-lamp. The carbon monoxide from the fire killed those men that were in that mine. Many of course escaped.

Here we have one hazard, a fire hazard. Now we have another one, a health hazard due to the toxic materials. We have to evaluate them. Certain of these materials, I am informed, are also flammable. I am not just sure how flammable they are but flammability probably decreases as your chlorination increases. But your toxicity also increases as your chlorination increases, according to Dr. Drinker, and I think that checks with other findings. We have these hazards to balance but we can control them. I feel quite confident that we can.

Perhaps we can find other substances that are better even than this material. At the present time I am advised that we have no better. I am advised that in ships, such as submarines, it is quite important to have a non-flammable substance for the insulation of their electrical wire.

DR. CECIL K. DRINKER: Dr. Gray, would you care to say a word?

DR. ALBERT S. GRAY (Director, Bureau of Occupational Diseases, State of Connecticut Department of Health, Hartford, Conn.): Dr. Drinker, I don't think there is anything that I can say except that we used this material in Connecticut and we had a

number of so-called cases. All I can say is a confession of ignorance on our part at that time and I was very glad to see that your digging into the literature as thoroughly as you have hasn't elicited any further information than we were able to get, basically. About all we could find was about the two cats that our friend, Lehmann, experimented with. We got in touch with him and he confessed that the amount that he had given the cats and his method of determining it were probably subject to very serious scientific criticism, and we agreed with him on that point.

We felt, and I am very glad that it is what you feel as a result of your experiments, that if we put in an efficient ventilation system sufficient to prevent the dermatitis, we probably wouldn't have liver damage. That is what we did entirely empirically.

I think that is about all I have to say. I have been delighted to be here and to have as many members of my staff here as I could jack out of the office. We have been delighted to listen to the discussion.

DR. CECIL K. DRINKER: May we now make the discussion general and those who have questions please volunteer.

MR. MANFRED BOWDITCH (Director, Division of Occupational Hygiene, Massachusetts Department of Labor and Industries, Boston, Mass.): I would like to second very strongly what Dr. Sayers has said with regard to the approach to this problem in proper control rather than by prohibition, which seems to me wholly unnecessary not only in this case but in the case of other toxic substances with which we have to deal. The problem of proper ventilation seems to me relatively simple from what I have heard of the situation thus far. The problem of maintaining that ventilation properly does not seem to me quite so simple. I think it is going to be quite necessary that there should be a very adequate inspection of the plants using the materials and I can assure you that that will be the case here in Massachusetts.

When this trouble first broke in this state a little over a year ago we issued a quite strongly worded warning letter to all plants using these substances and it has all along

been my plan to follow that up with a further letter bringing the situation as we now know it, as a result of Dr. Drinker's work, to the attention of users in this state. In that letter we shall unquestionably advocate the concentrations that Dr. Drinker has suggested this morning.

One point that I think is worth commenting on in that connection is the question of identification of the substances. It is extremely important from the state administrative point of view that employers using these substances should know what they are using. They are identified at present only by numbers. If we list those numbers in a warning letter which we send out there are likely to be changes in those numbers. The Halowax Corporation is a progressive concern and it is going to be putting out bigger and better halowaxes all the time. The company's cooperation in this present investigation has been such that I feel assured that they will take the proper steps to see that we in the state offices and also the users of the products will know what we are using.

I wonder whether Mr. Brown would care to say anything about that.

MR. SANDFORD BROWN (President, Halowax Corporation, New York, N. Y.): There are some aspects of this situation on which I think I can enlighten the medical and state and civic authorities, with respect to the commercial and practical aspects. If you go into the research laboratories of any large chemical manufacturing company today you will find anything from one to a thousand different new chemical products which have not yet been put on the market. Some of those may or may not be toxic. The problem so far as the chemical manufacturer is concerned is a question of timing. You have heard this excellent presentation given this morning by the Drinker brothers as to the work that they have done here. Should you take a product of which you have developed, say, 5 or 10 gm. and spend \$50,000 on research to determine whether or not it is toxic or should you wait until you have determined whether you have a market for it?

If you are producing only one hundred substances a year you can see that that would run into boxcar numbers in the way

of dollars and cents before you ever sold any. That is the problem we have had in this case. It has been on the market for 25 years. Until within the past 4 or 5 years there has never been any intimation that it would cause any systemic effects. Thousands and thousands of workmen have dealt with millions and millions of pounds of certain of these materials, particularly the trichloronaphthalenes. Then we come to the higher stages, combined with chlorinated diphenyl and other products, and suddenly this problem is presented to us.

We had asked various authorities interested in public health, going back over a period of 15 to 20 years, to investigate it but there wasn't much enthusiasm for it. Mr. Bowditch suggested that we take it up with the Drinker brothers at this institution, which we did. You know the results of that work.

Now so far as these changes are concerned, they are beyond our control to a certain extent. We will manufacture a product—let's call it 1234. It has certain chemical and physical characteristics. It is supplied to a cable manufacturer. It is composed of certain constituents. There may be some tetrachloronaphthalene, trichloronaphthalene, paraffin, a little pitch or bitumen, and possibly some chlorinated diphenyl. It does a certain job but he wants it to a little more plastic or he wants its viscosity or the specific gravity changed. Possibly by a change in those constituents of only 1 per cent we can get that particular property. We can't sell it to him as the same product so we put a new number on it. Basically, however, if the variation in the chlorinated naphthalene or the chlorinated diphenyl constituent hasn't changed, the toxic property of that will not change.

When it comes to a question of cooperating with the state authorities in that connection, if there were some major change made in a product we would have no hesitancy in advising them and we would also advise our customers. Virtually every consumer of these materials at some time or another has been given their technical or chemical designation along with their various properties, whether they be physical or chemical.

Those are some of the practical problems

with which all manufacturers of chemicals are confronted today, particularly in the synthetic organic field where the development is so rapid that our sales departments can't even keep up with the research departments sometimes, in knowing what they are doing.

Mr. F. R. KAIMER (Assistant to Manager, York Wireworks, General Electric Co., York, Pa.): I am certainly pleased to have this opportunity to say a few words with reference to the experience we have had at our York plant. I perhaps should say that again in this case experience alone has been the best teacher. I have lived with the problem at York with the men who went through the experience from its beginning.

It is only 1½ years ago that we had in the neighborhood of 50 to 60 men afflicted with various degrees of this acne about which you all know. Eight or ten of them were very severely afflicted—horrible specimens as far as their skin condition was concerned. One man died and the diagnosis may have attributed his death to exposure to halowax vapors but we are not sure of that. There was an atrophied condition found as a result of the autopsy but we are not definitely sure that it was or was not connected with his work. Knowing the man as I did when he was employed, with the superficial examination that he had, he appeared to me very thin, pallid in his appearance, and I would not say from my poor knowledge of the physical make-up of the human being that he presented a healthy appearance. However, it was only for a 6 months' period from the time of his employment that he complained of this constipated condition and we advised that he see his home physician. It rapidly developed to the point where he was in the hospital and in a very short time he died.

More serious than that perhaps is the fact that we had 50 other men in very bad condition as far as the acne was concerned. The first reaction that several of our executives had was to throw it out—get it out of our plant. They didn't want anything like that for treating wire. But that was easily said but not so easily done. We might just as well have thrown our business to the four winds and said, "We'll close up," because

there was no substitute and there is none today in spite of all the efforts we have made through our own research laboratories to find one.

But we did develop—and I was most closely associated with it—and set up a routine for bringing these men back to normal health conditions. A number of them were sent to Dr. John H. Stokes and to Dr. O. H. Perry Pepper of the University of Pennsylvania Hospital, and the others to Dr. Isaac R. Pels at Johns Hopkins. Through their recommendations and studies we employed a trained nurse and two local physicians and you might say established a small hospital and its facilities at the plant. Through the application of quartz light, x-ray, mechanical removal of comedones and the treatment of pustules that developed in later stages, an utterly strict routine where the worst cases were administered to each day for a period of 15 to 20 minutes, another group who were less serious three times a week, and still another group once a week, we have in this year and a half brought each and every man back to a normal skin condition. Those who were very seriously afflicted do show scars, but otherwise their skin is as healthy in appearance as my own. I tell you we are very proud of the fact that they are still all employed and the amount of halowax that we are using today is even greater in quantity and in types than we were using a year and a half ago.

With the adequate ventilation system we have installed, with the routine for change of clothing from street clothing to work clothing when they come to work and the reverse of that process, with the assurance that a shower will be taken before the street clothing is again put on, we have found no recurrence of this skin trouble. Each and every man working with halowax products, either from solution, from solid compounds or handling the wire insulated and treated with it, is examined twice yearly with a complete physical examination, including blood analysis and efforts to determine any liver damage.

However, there is the point which was very definitely brought out this morning. We do not know as yet when this thing starts. I believe it would be of great help

to us all in industry if some very fundamental work was done on humans, as Dr. Jones mentioned before we came into this meeting, some control in fundamental work to determine some method whereby we may determine the inception of any liver disorder.

We have had our men examined in groups three times since the trouble developed and nothing has shown up in any one man. We have learned and appreciate the fact that we must handle hazardous materials, but we have learned how to handle them. It has been a very great experience for us and for myself, and again the best teacher.

DR. CECIL K. DRINKER: Can you recollect the air concentration that you are maintaining now with your present ventilating equipment?

MR. F. R. KAMER: We have had two examinations made of the air by Mr. William F. Hemperly and they average from 0.2 to 0.6 mgm. per cu. m. We recently discovered one condition which was found as the cause of a high reading in one of the examinations. The compound is one that is not manufactured by the Halowax Corporation that is used rather infrequently, perhaps not more than 50 gallons of it in a 6 month period. It is in the form of a paste, reduced with solvent to a paste form, and it was not known that it contained a chlorinated solvent in certain percentage. We discovered it only recently through the analysis of a sample and we found that that was the cause of throwing the results off to a high value. It was then about 8 mgm. per cu. m., I believe. That is going to be eliminated now. That is a combination of toluene and carbon tetrachloride solvent. When that compound is exposed to the air the chlorinated solvent odor is completely masked by the toluene. It is lost. I was able to detect it only by removing the head of the drum and immediately smelling the odor of carbon tetrachloride. As soon as that head was removed for a period of 10 seconds the toluene predominated and we had no knowledge of the other being in there.

MR. ARTHUR G. BERN (Research Engineer, United American Bosch Corporation,

Springfield, Mass.): Do I understand you to say that those men who were afflicted are back on the same job?

MR. F. R. KAMER: They all returned to normal skin health, as I say, and are working on the same job.

DR. CECIL K. DRINKER: Dr. Schwartz, you have seen a good deal of this general subject.

DR. LOUIS SCHWARTZ (Medical Director, Dermatoses Investigations, United States Public Health Service, New York, N. Y.): I am primarily interested in dermatitis and it was only because of my researches in that field that I happened to come across information given to me by Dr. Gray that there were some cases of yellow atrophy of the liver attributed to halowax. I can only talk from personal experience about the skin cases. I know from what I have seen and from what I have heard from doctors who have been treating these cases that if they are treated like an ordinary acne vulgaris and kept away from further large exposure they all get well, with the same results as acne vulgaris. Those who have large pustules may have scars resulting and those who have superficial pustules or only comedones won't have any scars.

As far as prevention goes, I felt even before this investigation was undertaken that this substance, like any other poisonous substance, can be handled and used in industry provided proper safety precautions are taken, and I so advocated at the meeting in Pennsylvania when this was discussed.

About ventilation and safety precautions, I think that while we cannot, with our present knowledge, detect by any chemical tests the early symptoms of intoxication from this substance, the skin offers an easy way of proving whether your method of ventilation is efficient or not. If there are any cases of acne or of this dermatitis occurring in a plant where halowax or the chlorinated naphthalene or chlorinated diphenyls are used, then that shows that there is sufficient concentration of these substances in the air to cause plugging of the follicles and to cause a skin condition. If there is sufficient concentration to do that there may be

sufficient concentration to cause systemic poisoning in the few people who are hypersensitive to the action of these hydrocarbons. Everybody is not hypersensitive to them. If they were, of the thousands of people who have been handling these substances, more would have contracted this disease and it would have been reported very much earlier. This material was used in Germany as early as 1914 or 1915.

While I cannot add anything to the methods outlined here for safety in the way of ventilation and so forth, I can say that if you go into a plant and find that there is any acne present among the workers with this material you will know that there is sufficient of the substance in the air to cause acne and consequently sufficient perhaps to be dangerous to the few people who are hypersensitive to its action.

DR. CECIL K. DRINKER: There is a practical point in regard to the question of determining these substances in air. My brother pointed out this morning that there are several pieces of apparatus for doing this. It has seemed desirable to a number of the men who are here that during the next couple of years, while we are in a position of learning, that we endeavor, if we can, to confine our analyses to one piece of apparatus, so that at least our results will be as comparable as we can make them from place to place.

I don't know much about this phase of the subject; perhaps, Mr. Cook, you have an idea or two about that.

MR. WARREN A. COOK (Chief Industrial Hygienist, Bureau of Occupational Diseases, State of Connecticut Department of Health, Hartford, Conn.): On the determination of the halowax I wonder if it wouldn't be preferable to have Mr. Hemperly discuss the method he has been using throughout the country for the determination of chlorinated naphthalenes.

DR. CECIL K. DRINKER: I think so far as that is concerned it is best to leave that to discussions with Mr. Hemperly and Dr. Brandt afterward. But the question of determining some uniformity of technic for a while at least is, I think, a matter of general interest to us.

MR. WARREN A. COOK: I am not quite sure that I have in mind exactly what you want in that regard. In connection with sampling?

DR. CECIL K. DRINKER: Yes, In connection with the whole technic of determining the material in air, length of sampling, type of apparatus, and so forth. It has been suggested that we endeavor to agree upon some uniform procedure for that and as figures begin to come in, as they will in the course of the next couple of years, we will at least have them on a comparable basis.

MR. WARREN A. COOK: In that regard there is no question but what the determinations and results of analyses of the concentration of injurious materials in air, correlated with pathological findings or freedom from pathological findings, are real factors in further substantiating toxic limits which are originally given us from animal experimentation. In order to obtain data which are of value along that line it is desirable to know to what extent, in terms of milligrams per cubic meter, workers are exposed to in breathing any potentially injurious material.

It seems to me that one of the things that should be done, which many of the plants and many of the organizations equipped to make determinations of injurious materials in air should do, is to make an effort to develop one group and series of results which will represent average exposures of workers to chlorinated naphthalenes. In getting that figure I think that it is of great importance that we should consider average daily exposure. There is a tendency very often in taking samples of air to determine maximum exposures, to get the exposure over a 15 or 20 minute period when the worker may be actually tending a pot where the material is being used, whereas during the subsequent half hour he may be 40 or 50 feet away from that particular location. Therefore, for one thing, it seems to me it would be extremely desirable to obtain information in as many plants and as many locations as possible of the average exposure, taking into consideration not only the worker's maximum exposure while actually

at work with these materials but also his much smaller exposure and possibly even lack of exposure as he may go to other parts of the room or even to other departments during the course of the day.

Another type of determination which seems to be extremely desirable to have is concentrations of the chlorinated naphthalenes at certain reference points. I believe that Mr. Hemperly very carefully duplicated these reference points in each of the plants where he took his determinations. He took the samples, I believe, at breathing level and a certain measured distance from the pots where the chlorinated naphthalenes were being handled, so that next year he could go back to those same locations or others could take determinations at those same locations, which are definitely fixed and recorded, and learn whether, due perhaps to clogged pipe lines or to fans which might become loaded with the condensed chlorinated naphthalene, there might be poorer ventilation than originally designed and higher concentrations of the chlorinated naphthalene than originally determined.

It seems to me that it would be an extremely desirable thing to have made available for all of us who are making these determinations these reference points so that in Massachusetts and in Connecticut, at one plant or at another, a considerable group of workers could be taking samples at this one particular point with reference to the source of generation of the vapor. Such results would permit a comparison of knowledge between plants.

In that regard it happens that there is a committee of the American Public Health Association which is charged with the development of chemical methods for determination of atmospheric contamination such as that now under consideration. It seems to me that it might be a very desirable project for that committee to consult with the investigator of the Halowax Corporation and suggest to all of those who are doing work of this type, after some discussion, what reference points could be adopted and also what should be done in connection with getting these average exposures. As a matter of fact our committee will discuss that very feature and possibly include it in our report

before the A. P. H. A. in October as one of the projects which we shall carry out at that time.

As I have not given this consideration, I am afraid my discussion is not as connected as I might wish it to be. We have a real problem here and one that deserves some study.

DR. CECIL K. DRYER: Would it be of service to the American Public Health Association Committee if a sub-committee from this meeting, collected perhaps by my brother and those of you involved with these problems, were to make a recommendation to the A. P. H. A. as to the things you have brought up and as to suitable apparatus, and let the final statement come from the Association after they have considered that material?

MR. WARREN A. COOK: I think that would be a splendid idea because we have a bulk of information on this particular material. A number of individuals have given much thought to the development of apparatus, both the combustion method which we have here and also the continuous record type of apparatus which Professor Drinker mentioned this morning. I think that it is a sufficiently important project that a sub-committee made up from this group could give that committee a very definite steer.

[Personnel of the Sub-committee: W. A. Cook, chairman, A. L. Coleman, H. B. Elkins, W. B. Fulton, S. W. Gurney, E. R. Hayhurst, W. F. Hemperly, F. W. Sehl, and A. N. Setterlund.]

MR. HERVEY B. ELKINS (Chemist, Division of Occupational Hygiene, Massachusetts State Department of Labor and Industries, Boston, Mass.): In connection with what Dr. Schwartz has said, I am not sure that I am in complete agreement as regards the use of skin lesions as warning agents. It is my understanding that in insulated wire plants the chlorinated diphenyls are very commonly compounded with the chlorinated naphthalenes. It is also my understanding that these are more likely to cause a severe skin irritation than the straight chlorinated naphthalenes. On the other

hand, from Dr. Drinker's work one would judge that the straight chlorinated naphthalene was nearly as toxic at least as the mixture of it with a small amount of chlorinated diphenyl.

In the condenser factory where we had our cases there is no chlorinated diphenyl used and no acne of the type described in the reports on the insulated wire factories.

Dr. R. EMMETT KELLY (Monsanto Chemical Co., St. Louis, Mo.): I can't contribute anything to the laboratory studies but there has been quite a little human experimentation in the last several years, especially at our plants where we have been manufacturing this chlorinated diphenyl. It has been our observation that although on one occasion we did have a more or less extensive series of skin eruptions which we were never able to attribute as to cause, whether it was impurity in the benzene we were using or to the chlorinated diphenyl, we have never had any systemic reactions at all in our men. We have examined them very closely both from what laboratory tests we thought might help us and from the clinical viewpoint. Also from chlorinated diphenyl alone there have been no cases of systemic poisoning reported.

I don't believe that we can transpose the laboratory results into the actual humans without paying considerable attention to the volatility of different substances and the way they are being used.

Dr. JOHN A. HOOKER (Consulting Dermatologist, Halowax Corporation, Detroit, Mich.): I have had considerable experience with the dermatological angle of this problem. The experience at the Halowax plant at Wyandotte definitely was that the dermatosis did increase when compounds were used rather than the straight chlorinated naphthalenes.

From the discussion we have heard today I think the observation Dr. Drinker made that the chances are that dermatological problems will disappear along with the other is probably true. However, I think that certain precautions, certain insurance measures, you might say, should be taken in hiring men who are going to work with these products, even though we are going

to attempt to get the concentrations low. We saw this morning that the effect of these products was on the liver and that if some one working in these products developed liver damage from some other source the chances of his having serious trouble was increased. Therefore, inasmuch as there is no test to tell whether a person has had liver disease, certainly in the preliminary medical examination of applicants for work the history at least should be gone into very carefully for a history of previous liver diseases.

There are other things that should be taken into consideration also. One is the history of syphilis, because syphilis does have an effect on the liver. If there is any history of syphilis I don't think that that man should be hired. A Kahn test should be taken and I think that it would be a good plan to take Kahn tests on men at stated intervals, because they might develop syphilis after they are hired.

I think those things should be done even though we feel that if the concentration of these vapors in the air is kept as low as was recommended this morning, the chances of having toxic effects are very small.

Mr. F. R. KANZAR: I would like to clarify a point of Dr. Hooker's.

You mentioned at the beginning of your talk that your experience in acne condition was more pronounced from the compounds of various materials with chloronaphthalene than with the straight chloronaphthalenes. Did you mean by that, that the acne condition actually was more pronounced with the compounds than with the straight chloronaphthalene or was that due to the fact that you were in a plant operated in a different fashion? In other words, you handled your numerous wire compounds in more of a batch operation. That is my understanding from Mr. Brown.

Is it a fact that the straight chloronaphthalenes are less harmful in so far as acne is concerned or is it because of the handling of batch compounds?

Dr. JOHN A. HOOKER: That is a question that would be difficult to answer. I based my statement on the fact that in taking a general survey of these cases after I started working with them the men in the plant said

that they developed acne more often and more severely after they started making compounds. It may be that it was in the method of the handling.

MR. F. R. KARMEN: You say that the condition increases with the increased chlorine content and we are attributing the acne primarily to the chlorine radical. If that is true, what are we to associate insofar as these various other constituents are concerned, such as various plasticizing agents? How much do we know about those insofar as development of acne is concerned? Some of these compounds have a half dozen constituents in them other than chlorodiphenyl and chloronaphthalene. We are at the present time considering the chloronaphthalene as the basic element responsible for the acne condition which we have. We know very little about what the other materials may or may not do.

DR. JOHN A. HOOKER: I don't know about these other materials. Perhaps Dr. Schwartz would know something more about them.

DR. LOUIS SCHWARTZ: It is well known in industry that there are many compounds that cause acne. For instance, paraffin acnes and oil acnes have been known for a long time. Paraffin acnes, wax acnes, are simply a mechanical plugging of the follicles of the skin by the minute particles of wax that fall on the skin. I think that the acne caused by these chlorinated naphthalenes and diphenyls, because it is caused by both—I was down at the Swan Chemical Company where they make chlorinated diphenyls and they have acne there just the same as over at Wyandotte where they make the chlorinated naphthalenes—I believe that that is caused by the same thing, a blocking up of the follicles of the skin, the formation of the comedone and then the comedone becomes infected and forms a pustule. They don't all pustulate because they don't all become infected. Many of these men never have any pustules. They just have little white elevations on their skin.

In those people who are in the acne age, the youngsters of 13, 20, 22 and 23, acne

vulgaris is most apt to occur. The sebaceous glands are most active and they seem to have it much more than the older people in whom the sebaceous glands are not so active.

We keep a clipping of all articles relating to industrial dermatitis in my office and I recently read an article in a German magazine in which they report in Germany a similar acne condition from chlorinated phenols and chlorinated benzenes and solid waxes. It seems that this acne-like condition is not peculiar to halowax or chlorinated naphthalenes or diphenyls but can occur with any condition that blocks up the sebaceous follicles of the skin.

DR. ROYAL MEEKER (Special Agent, State of Connecticut Department of Labor, Hartford, Conn.): I am full of questions. As a statistician I am a little bit suspicious of averages, so I should think that we should take maxima as well as averages in getting at the threshold dose or the dangerous impregnation of the atmosphere with these poisonous compounds. I just wanted to drop that as a hint for this committee to consider in its work.

In spite of what you said at luncheon, Dr. Drinker, I still think it is rather important to determine how the plugging of the pores actually does occur, whether they are plugged as Dr. Schwartz has suggested, from the particles falling on the outside of the skin or whether they are plugged, as so many doctors have informed me, from inside as it were. The fumes are breathed, the substance gets into the blood stream. It is thrown off through the pores of the skin and the pores are plugged. I think it is rather important to determine that because it seems to me, from the slight knowledge that I have of the industrial application of halowax compounds in insulating wires, that the plugging of the skin, if it is just an outside plugging, may take place either from handling a solid substance or from the coating on the wire after it becomes solid.

I haven't heard anybody say anything about those Massachusetts cases of very severe dermatitis and I am not so sure but what there was some question of liver damage there. Nobody has said anything about them at all. There is no question there

that the condition, whatever the complications might have been, came from handling cold wire, in a confined area of course.

I think this needs a great deal more study, Dr. Drinker, than you and your associates have been able to give it as yet. All I am curious about is the way we can administer the protective labor laws in order to protect both the employer and the employee.

I listened with very great interest to the expositions of suggested pots for the application of halowax, chlorinated naphthalene and diphenyl. It seemed to me that they left very much to be desired. I don't see why the application of the halowax compound, if it is necessary, as I assume it is, to use this rather dangerous substance, can't be done in a practically closed pot. If you please, Dr. Drinker, I wish you would ask Mr. Reeves of the Rockbestos Products Corporation to tell how they have handled it. If I am any judge of equipment and safety devices they have come as near to eliminating entirely from the atmosphere all contamination from fumigated or evaporated halowax as anyone. All of the application of halowax, either in the melting pots or where it is applied to the wire, is done under hooding and with ventilating apparatus, and it seems to be entirely adequate.

Mr. B. H. REEVES (Vice-president and General Manager, Rockbestos Products Corporation, New Haven, Conn.): I will be very glad to tell of our experience if it will help the picture along. Our experience has been more or less parallel with Mr. Kainer's in as much as we manufacture the same type of wire. We have avoided the serious picture that he ran into, probably due to the fact that shortly after 1923 we were using benzene as a solvent for asphaltic materials.

We got into anemia difficulties with benzene fumes and had to install a complete ventilating system to remove all those fumes from the machines that applied the compounds to the wire. Following the installation of that equipment we were able to get a substitute for benzene, so we got it out of the picture.

We are still in the same fix as every one else is as far as finding a substitute for halowax. There is none that we know of. Consequently when we went to the use of halo-

wax we had a head start as far as the ventilating system was concerned, since ours was already installed and adaptable to a compound with a benzene solvent rather than a melted halowax compound. In order to keep the fumes of a highly volatile solvent under control we did develop a practically complete enclosure for the container in which the compound was placed and exhausted that container in order to prevent any escape of the fumes from it. When we changed to the halowax compounds we followed the same scheme of applying the compounds and used a completely enclosed pot. There is a hole in one side for the wire to go in and one on the other side for the wire to come out. There is enough exhaust suction on the enclosure to prevent any fumes from getting out of the openings where the wire enters and leaves. Of course periodically it is necessary to open the pot to replenish the compound or make adjustments. In that case we have the ventilating system interlocked with the door that the operator opens so that when he opens it he increases the amount of air travel about ten-fold.

Dr. ROYAL MEEKER: What is the velocity when the door is open?

Mr. B. H. REEVES: With the door wide open, at breathing level the velocity is 200 feet. That is the linear velocity. We check that every week on every opening at every machine with a velometer, a small instrument which is easy enough to operate and reads directly. Whenever the velocity falls below that figure the pipe connecting that machine is taken down and cleaned, because there will be condensation inside the pipe to cut down the air flow and reduce the ventilation efficiency.

Dr. ROYAL MEEKER: What do you think of that trap device shown?

Mr. B. H. REEVES: I don't know what to think of that. I don't know whether it would be practical or not. I was going to ask Dr. Drinker whether in passing the air laden with halowax fumes through the boxes of rats there was any deposit of halowax in the enclosure.

DR. CECIL K. DRINKER: Yes. It is detectable readily through the outflow pipe.

MR. B. H. REEVES: Those in the far end probably didn't get the concentration.

DR. CECIL K. DRINKER: We change the position of the boxes each day. The ones furthest away move into the nearest position, so that over a period of weeks all are exposed equally.

DR. EMERY R. HAYHURST (Consultant in Industrial Hygiene, State Department of Health, Columbus, Ohio): I would like to ask if it wouldn't be possible to put a cold water line around that trap and deposit it all before the air escapes from the trap.

MR. B. H. REEVES: We have tried cooling the surface of the exhaust pipes in order to concentrate the deposit in one place and make it unnecessary to remove long lengths of the ventilating system. While we have found a slight advantage in doing so it hasn't at all paid for the expense. It is much easier to take the piping down. Consequently I doubt the possibility of removing it all by cooling the surfaces of the trap. It might be possible, by building a labyrinth of refrigerating coils, to collect it. But then you would have the problem of removing it from the coils after you had collected it and it would involve rather complicated procedure.

DR. B. L. VOSBURGH (Medical Director, General Electric Company, Schenectady, New York): We have heard about the possibility of preventing liver damage and skin trouble among manufacturers of wires and cables and so forth, but like the old rhyme, every dog has fleas and the fleas have fleas, we also have customers who use wires and cables in tunnels, in enclosed spaces, splicing them together and doing all sorts of things. I am not at all certain that we know what the concentration of these chlorinated hydrocarbons is under those conditions.

About the time we were having so much trouble at our York factory some of our customers began complaining. We thought we were having a hysteria of halowax mania throughout the country. Some of their

complaints were bona fide. We had no difficulty in handling cold wire and the men had no skin trouble. Yet when they did the same thing with possibly the application of heat in tunnels, ships and so forth, they actually did develop trouble.

We have used, of course, the interus index in following some carbon tetrachloride cases and I wondered what you have to say about that. Is that of no value at all in the case of liver damage in this material?

DR. CECIL K. DRINKER: I don't think so until it gets so extreme that we know it any way.

DR. B. L. VOSBURGH: I did write down a few questions that came to mind. Some of them have been answered and some are foolish but I shall ask them anyhow.

It occurred to me that if trichloronaphthalene has been proved to be practically innocuous, isn't it a problem of research largely for the Halowax Corporation to use only that one material adopted with other known chlorinated compounds to meet the requirements of customers? That is one of the foolish ones probably, but I shall just ask it.

Furthermore, how long is it reasonable to expect the liver to recover after it has been pretty well damaged. I talked to you and you said you hadn't worked that out with the animals as yet.

DR. CECIL K. DRINKER: In that case we set aside a certain number of animals to recover, after knowing that a certain amount of damage had been done. Then we started to kill them but we invariably ran out of rats before they ran out of lesions. We don't know how long it lasts.

DR. B. L. VOSBURGH: Have you given them calcium and glucose as you would with carbon tetrachloride?

DR. CECIL K. DRINKER: Not yet, but we are doing an experiment now with one group on a very high calcium diet and another group on a calcium free diet. The whole question of calcium in the diet, of giving milk daily to these people may be worth while.

Dr. B. L. Vossbach: I just had those questions in mind. I would like to hear what Mr. Brown would say as to the possibility of using only this one innocuous chlorinated naphthalene.

Mr. SANDFORD BROWN: That would simplify our problem exceedingly, not only from the commercial standpoint but from the toxicity standpoint. But if we did so I think that Mr. Kaizer would be the first to object because he wouldn't be able to get the technical effects he desires in the cable. The same thing applies throughout all the industry where we are using these higher chlorinated products.

The problem as we see it, and it is one which we intend to study further (we have our program for this continuous work that will be done under the direction of the Drinker brothers), is to try to find a line of demarcation and whether or not within this complicated chemical structure there is some one thing that causes the trouble. If we could take hexachloronaphthalene and remove 1 per cent of some unknown constituent with which we are not now familiar and eliminate the toxicity, that would be a lovely solution. Whether we will ever be able to attain that I don't know. That is one of the things we have in mind.

As I said before, that is one of the things with which all manufacturers of these synthetic organic products are confronted at some stage, either in their laboratories or in commercial developments. It is largely a question of timing as to when you should do the work, how much you should do, and whether you know how to do it. You have to learn that first.

Dr. CECIL K. DRINKER: Unless there is some other important matter I will declare the meeting adjourned.

Dr. R. R. SAYERS: May I say a word more with regard to what Dr. Hookey stated on syphilis? If you are going to eliminate all your workers who happen to develop

syphilis during the time they are working for you, you are probably going to lose some very important employees. It would seem that it would be worth while to give serious consideration to seeing that conditions are proper and suitable for them to continue working for you and that they have proper treatment. In other words, I don't believe that is a good or sufficient reason for discharging those employees who do develop syphilis. We have not found that to be true in other places and I question whether this is one of the places. I may be in error.

Mr. SANDFORD BROWN: I want to take this occasion to express on behalf of the Halowax Corporation my thanks for the excellent cooperation we have received from various state authorities and our customers as well in this investigation. I believe you have all formed the opinion, based on what you have heard this morning, that our whole motive here is to conduct this on an ethical, scientific and constructive basis.

In collaboration with the Monsanto Chemical Company we have a much more comprehensive program in view to carry on. Therefore we want to continue that same type of cooperation.

There is one thing that I want to bring up in that connection, which will have to be observed by the state authorities, when they go out to make these inspections. That is the necessity of not creating mob hysteria on the part of the workmen in the plants where these inspections are made. Mr. Hemperly has run into some very interesting situations in the various examinations he has made. I know that he would be glad to relate them and give the benefit of his experience to the heads of any of these state departments who will have the direction of this work. Otherwise I can see where we will be unable to get the full cooperation of not only the individual workmen but the plant foremen and the management, and we must have that if we are to get the results that we are shooting for over the next several months or years. This thing may continue, probably will continue for years.

MORPHOLOGICAL CHANGES IN THE LIVERS OF RATS RESULTING FROM EXPOSURE TO CERTAIN CHLORINATED HYDROCARBONS*

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CHLORINATED hydrocarbons, particularly chlorinated naphthalenes and chlorinated diphenyl, have been used extensively in certain industries. Their use in the manufacture and preparation of many types of electrical equipment is constantly increasing. Although it is known that some of these compounds cause acne, only recently has the possibility of more serious systemic effects been recognized.

During the spring of 1936 we were informed of the occurrence of three fatal cases of jaundice in workmen using chlorinated naphthalenes and chlorinated diphenyl. At the request of the manufacturers of these compounds, we undertook an investigation

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This is the second of three papers read at a symposium on chlorinated hydrocarbons given at the Harvard School of Public Health, Boston, June 30, 1937. The third paper will appear shortly.

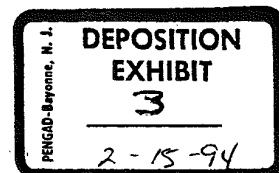
to determine what systemic effects, if any, would result from the administration of a number of these compounds to experimental animals.

The known findings in the three fatal cases of jaundice, together with a review of the pertinent literature have already been reported (1).

The present paper describes the pathological changes observed in rats that had been exposed to various chlorinated naphthalene compounds and to chlorinated diphenyl.

MATERIALS AND METHODS

A detailed description of the apparatus and technic employed by the authors in this investigation has been published (1). White rats, maintained on a diet of Purina Dog Chow, supplemented by lettuce, eggs, milk, and cod liver oil, were used throughout the experiments.



The chlorinated hydrocarbons tested were the following:

Compound A. A mixture of tri- and tetrachloronaphthalenes.*

Chlorine content 49.4 per cent.

Compound B. A mixture of tetra- and pentachloronaphthalenes. Chlorine content 56.4 per cent.

Compound C. A mixture of tetra- and pentachloronaphthalenes plus chlorinated diphenyl. Chlorine content 43.5 per cent.†

Compound D. A mixture of penta- and hexachloronaphthalenes. Chlorine content 62.6 per cent.

Compound E. A mixture of penta- and hexachloronaphthalenes. Chlorine content 62.6 per cent.

Compound F. A mixture of 90 per cent penta- and hexachloronaphthalenes, plus 10 per cent chlorinated diphenyl. Chlorine content 63 per cent.

Compound G. Chlorinated diphenyl. Chlorine content 65.0 per cent.

These preparations were selected because of their relative importance in industry. It should be noted that they also represent a wide range of chlorination.

All of the above materials were administered orally in varying doses. Compounds A, D, F, and G were selected for the inhalation experiments.

* This compound contained only small amounts of tetrachloronaphthalenes. In this paper it will therefore be referred to as trichloronaphthalenes.

† This compound consisted of a mixture of compounds B and G but in addition contained two plasticizers which have been considered to be inert. Without these materials the chlorine content of this compound would be between that of compounds B and D.

Inhalation Experiments

We were most interested in the results of the inhalation experiments because they more nearly simulate the type of exposure to which the workmen are subjected (1). The inhalation experiments were carried out in air-tight wooden compartments through which air, containing the volatilized compound being tested, was driven by electrical blowers. With this apparatus we were able to test simultaneously the effect of four compounds. Eighty rats were exposed to each compound. The methods for determining the daily air concentration (mgms. per cu. m.) of the chlorinated hydrocarbon being used, as well as the rate of airflow have been described (1).

Compound A (trichloronaphthalenes) was administered by inhalation to two groups of animals. In the first of these experiments, the rats were exposed to low air concentrations (average 1.31 mgms. per cu. m.) 16 hours daily for 134 days. In the second experiment the concentration was increased to an average of 10.97 mgms. per cu. m. This 16 hour daily exposure was continued for 102 days.

Compound D (hexa- and pentachloronaphthalenes) was administered to three groups of rats. In the first experiment the exposure consisted of 16 hours daily for 134 days, with an average air concentration of 1.16 mgms. per cu. m. In a second experiment the average air concentration was 1.44 mgms. per cu. m. This was maintained 8 hours daily for 143 days. In the final experiment, the average air concentration was increased to 8.88 mgms. per cu. m. and exposure, 16 hours daily, was continued over a period of 52 days.

Compound F (hexa- and penta-chloronaphthalenes 90 per cent plus chlorinated diphenyl 10 per cent) was administered in low concentrations to two groups of rats. In the first experiment an average air concentration of 1.37 mgms. per cu. m. was maintained 16 hours daily for 134 days. In the second experiment, an average air concentration of 1.66 mgms. per cu. m. was inhaled 8 hours daily for 143 days.

Compound G (chlorinated diphenyl) was administered to two groups of animals in low concentrations. An average concentration of 0.57 mgms. per cu. m. was employed 16 hours daily for 134 days in the first experiment. In the second experiment (employing an average air concentration of 0.93 mgms. per cu. m.) the animals were exposed 8 hours daily for 143 days.

Feeding experiments

All compounds tested were employed in the feeding experiments. A weighed amount of finely ground material was mixed with rat food. This mixture was placed daily in one food container to supply the rats in a given cage. Thus each animal had an equal opportunity to ingest the compound supplied. As the number of rats per cage was reduced, the dosage supplied was proportionately decreased. Most compounds were supplied in large and small amounts.

Supplementary experiments were conducted on a smaller number of rats by feeding several of these compounds in small known amounts by stomach tube or by injecting them subcutaneously as suspensions in gum acacia.

Carbon tetrachloride and alcohol administration to experimental animals

Seemingly the incidence of acute yellow atrophy in workers exposed to chlorinated naphthalenes and chlorinated diphenyl is very low. This single fact is of some importance in that it suggests that certain individuals may be more susceptible to the compounds or that in these instances the liver damage may have been intensified by some other agent. With this in mind, groups of animals that had been exposed for varying periods of time to certain chlorinated naphthalene compounds and chlorinated diphenyl were subsequently given a sublethal dose* of carbon tetrachloride and ethyl alcohol by stomach tube (0.75 cc. of each per kgm.). This dosage did not result in a single death among the 16 control animals and the degree of liver damage produced was quite constant and never very great. Administration of carbon tetrachloride and alcohol to rats exposed to the more highly chlorinated naphthalene compounds and chlorinated diphenyl caused extensive liver damage and proved widely fatal. Therefore, the use of carbon tetrachloride and alcohol has been employed regularly on representative groups of rats from each inhalation experiment.

Examination of tissues

Animals were sacrificed for pathological examination after varying periods of exposure. Complete autopsies were performed. Liver weights were recorded. In representative animals

* One cubic centimeter of carbon tetrachloride plus 1.0 cc. ethyl alcohol per kilogram when administered orally to normal white rats causes a 14 per cent mortality (2).

from each experiment, all organs excepting the central nervous system were examined microscopically. The livers of all animals were studied microscopically. Tissues were fixed in Zenker's fluid, 10 per cent formaldehyde solution, and absolute alcohol. The routine paraffin sections were stained with eosin methylene blue. Other stains employed were hematoxylin and eosin, phosphotungstic acid hematoxylin (Mallory), Foot's modification of Bielschowsky's stain (reticulum), Best's carmine stain, and Iron reaction with ferrocyanide of potassium (Mallory). In addition, frozen sections from certain liver specimens were stained with scarlet red, Lugol's solution, and methyl violet.

RESULTS

Compound A. Trichloronaphthalenes

(a) *Exposure by inhalation to low concentrations (average 1.81 mgms. per cu. m.) 16 hours daily.*—The 80 rats

subjected to these conditions showed no ill effects. Autopsies were performed on groups of 3 to 8 animals after 37, 72, 105 and 134 days' exposure. There were no significant abnormalities in liver weights.* Macroscopically the majority of the livers appeared normal although an occasional one was paler than normal. Rarely slight mottling was observed. Microscopically, the liver cells often appeared slightly enlarged, more granular than normal, and occasionally they were vacuolated. These slight abnormalities and the presence of a rare mitotic figure suggested that very slight injury to the liver cells had occurred. There was no demonstrable increase in the above changes after the first exposure period (37 days). Occasional livers of rats exposed for the total period showed

* Calculations pertaining to the weights of livers in these and subsequent animals are based on data in H. H. Donaldson's book "The Rat," 2nd ed. The Wistar Institute, Philadelphia, 1924 (p. 211).

PLATE I

FIGS. 1 AND 2. Camera lucida drawings showing the portal areas of the livers of two rats that were fed large doses of a mixture of tetra- and pentachloronaphthalenes and chlorinated diphenyl. The changes illustrated in figure 1 occurred within 48 days after exposure was begun. One should note the marked accumulations of hyaline globules in the cell cytoplasm, and the marked swelling of liver cells. Increased numbers of mitotic figures were also present. Similar changes are apparent in figure 2 which was made from the liver of a rat exposed 125 days. Degeneration in the central portions of the liver lobules is also present.

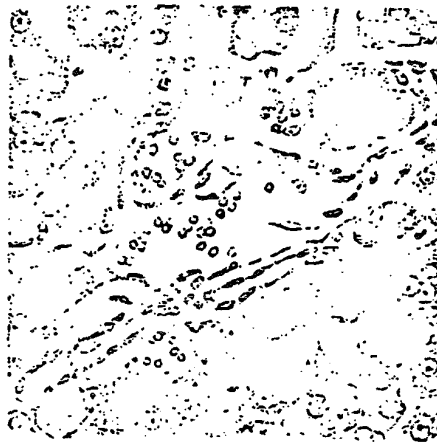
FIGS. 3 AND 4. These drawings illustrate the marked swelling and fatty vacuolization of liver cells observed in all rats that were fed small doses of tetra- and pentachloronaphthalenes. The changes shown in figure 3 were present after 26 days' exposure, those in figure 4 after 48 days' exposure. Note the mitotic figures in figure 3. The compound responsible for the changes contained no chlorinated diphenyl. Otherwise

it was similar to the compound responsible for the lesions illustrated in figures 1 and 2.

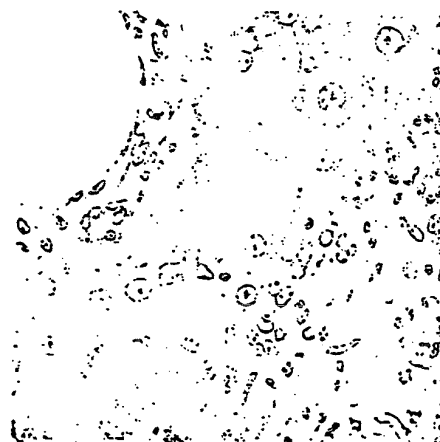
FIGS. 5 AND 6. Camera lucida drawings of the portal areas of the livers of two rats fed moderate sized daily doses of a mixture of penta- and hexachloronaphthalenes for 14 and 29 days respectively. In the first of these figures one notes swelling of liver cells with narrowing and distortion of the sinusoids. The liver cells show an increased granularity of the cytoplasm with a massing of basophilic granules near the nuclei. There is also an excess of fat in small and medium sized vacuoles. This was more prominent in the central portions of the liver lobules. An increased spacing between liver cells and between liver cells and sinusoidal endothelium is present. After 29 days' exposure (fig. 6) the above changes are greatly increased. Numerous large intercellular spaces containing serous precipitate, strands of fibrin and leucocytes had developed between liver cells. These spaces were bounded by distorted liver cells. See also plate III, fig. 1.

Camera lucida drawings $\times 395$.

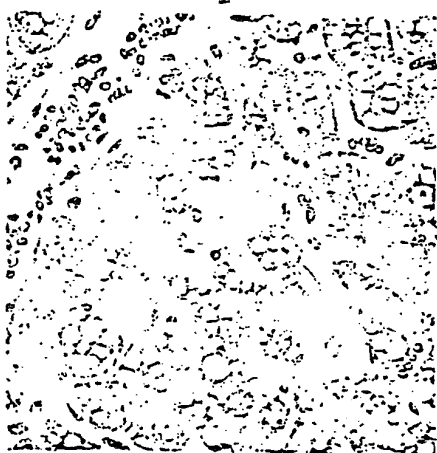
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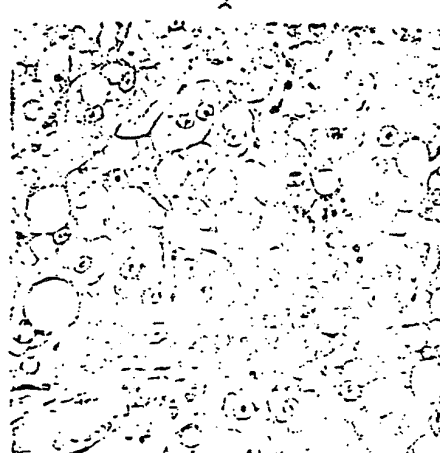
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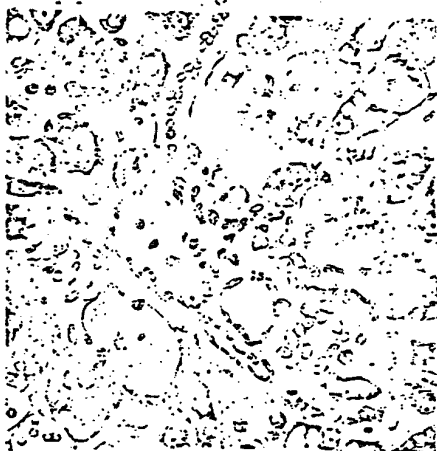
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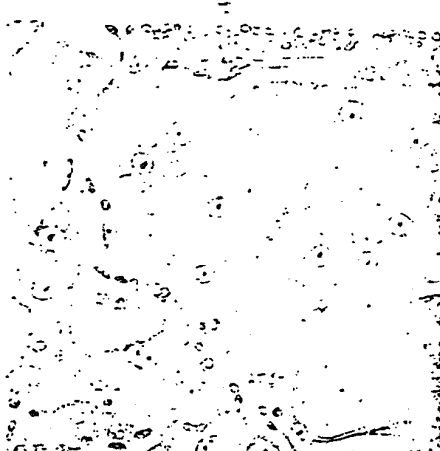
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no definite microscopic change. (See fig. 3, plate II.) All other organs were normal.*

Further evidence that the liver tissue injury had been very slight was obtained from the carbon tetrachloride and alcohol test. Of 10 rats exposed for 144 days in the above manner and then fed 0.75 cc. per kgm. each of carbon tetrachloride and ethyl alcohol by stomach tube, none died. At autopsy and on microscopic examination there was little or no evidence of liver injury greater than that produced by the same dose of carbon tetrachloride and alcohol in normal control rats. (See plate IV, figs. 1 and 2, and plate V, fig. 1.)

The livers of 8 animals exposed to trichloronaphthalenes for 105 days and autopsied after a 2 month recovery period showed no definite pathological change. This is further evidence that this compound is only slightly toxic. These findings represent a marked

contrast to the persistent hepatic lesions resulting from exposure to the compounds of higher chlorination.

(b) *Exposure by inhalation to high concentration (10.97 mgms. per cu. m.) 16 hours daily.*—In this experiment, the concentration of trichloronaphthalenes was approximately eight times that previously employed. Exposure was continued for 102 days. The 50 animals subjected to this concentration appeared normal. Groups of 3 to 17 rats were sacrificed after exposure periods of 31, 48, 59, 72 and 102 days. Most of the livers were pale yellow in color and slightly mottled. There was, however, no significant variation in liver weight. Microscopic examination revealed swollen liver cells with slightly increased granularity and vacuolization of the cytoplasm. Occasional degenerating and regenerating cells were observed. These changes, which were slightly more marked in the central portion of the liver lobules, were present after the first month of exposure. They

* In none of the experiments to follow were significant changes found in any organ other than the liver.

PLATE II

Figs. 1, 2, AND 3. Liver changes resulting from the administration of trichloronaphthalenes are illustrated in these camera lucida drawings. In figures 1 and 2 are shown the most marked changes observed after prolonged (100 and 136 days respectively) feeding in daily doses of 3 gm. per 10 rats. The changes observed consisted of slight to moderate swelling of liver cells, increased granularity of the cytoplasm and finally, after prolonged exposure, fatty vacuolization of the majority of cells and complete fatty degeneration of occasional cells. Figure 3 is a drawing from a section of the liver of a rat exposed by inhalation to low concentrations of trichloronaphthalenes for 105 days. No striking or constant changes were observed in rats so treated. Occasional cells are slightly swollen and show increased granularity of the cytoplasm. A rare mitotic figure was observed in occasional sections.

Figs. 4, 5, AND 6. Microscopic changes

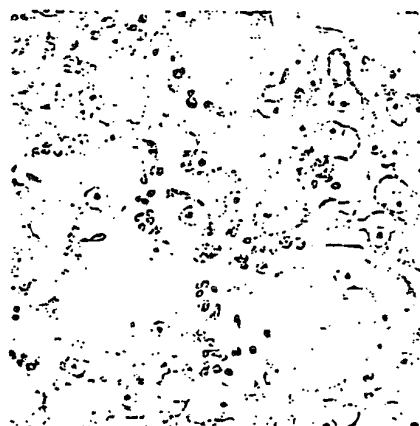
observed following the administration of a mixture of penta- and hexachloronaphthalenes are illustrated in these three drawings. Figure 4 shows the changes observed after feeding this preparation in 3 gm. daily doses per 10 rats, for a period of 30 days. The liver cells are markedly swollen and vacuolated. There is also necrosis and degeneration of scattered cells. Large intercellular spaces are observed. (See plate VI, figs. 1 and 2). Less marked but similar changes are observed in the livers of rats fed smaller doses of this preparation (see fig. 5). (28 days' exposure.) Figure 6 illustrates the liver cell changes that occurred after inhalation of low concentrations of penta- and hexachloronaphthalenes for 75 days. Note the swelling of the liver cells, the increased vacuolization and presence of large numbers of hyaline globules within cells. Mitotic figures were frequently observed.

Camera lucida drawings $\times 395$.

PLATE II



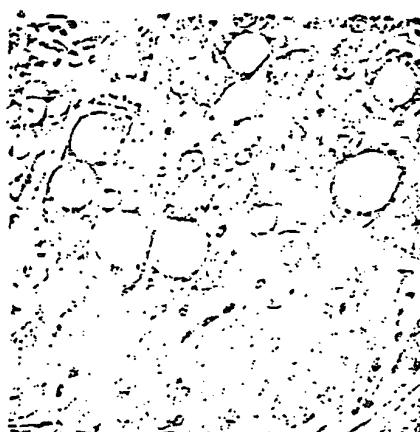
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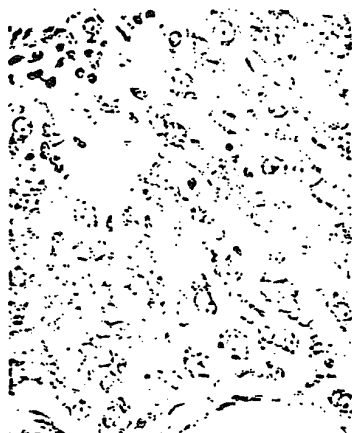
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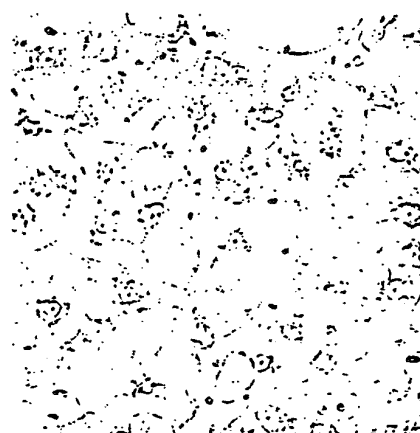
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were somewhat increased with longer exposure. After 102 days the liver cells were more markedly swollen and contained large and small fat vacuoles. Small hyaline droplets in the cell cytoplasm were occasionally observed. Mitotic figures, although never numerous, were increased in number. No structural changes had occurred in the liver lobules.

Carbon tetrachloride and ethyl alcohol were administered by stomach tube to 9 rats kept under these conditions. At autopsy their livers were slightly to moderately enlarged. They were yellow in color and the majority of them were mottled. The microscopic findings were variable. In one animal the observed necrosis was no greater than that seen after the administration of a similar dose of carbon tetrachloride and alcohol to normal control rats. The livers of 4 animals showed increased fatty degeneration in the central portions of the lobules with small necrotic foci. In the 4 remaining animals extensive central necrosis had occurred. In the

most marked of these (fig. 2, plate V), the central one-half or two-thirds of each liver lobule was necrotic and in occasional areas two or more adjacent lobules had undergone degeneration. Hemorrhage and extensive leucocytic infiltration had occurred in these necrotic areas. The less damaged liver cells at the periphery of the lobules showed large numbers of mitotic figures, indicating accelerated regenerative activity.

(c) *Exposure by feeding.*—Nine of the 10 rats fed trichloronaphthalenes in doses of 3 gm. per day per 10 rats were sacrificed at varying periods of time from 9 to 136 days. The tenth rat died of a respiratory infection after 182 days' exposure. There was no significant abnormality in the weights of the livers of these animals. After 2 months' exposure, microscopic examination of the livers showed slight swelling of liver cells. This was accompanied by increased vacuolization of the cytoplasm due to the accumulation of abnormally large

PLATE III

FIGS. 1, 2, AND 3. Liver changes resulting from the administration, by feeding and inhalation, of a mixture of penta- and hexachloronaphthalenes (90%) and chlorinated diphenyl (10%) are illustrated in these drawings. Figure 1 is a drawing made from a section stained with P.T.A.H. In addition to other changes it demonstrates the presence of large and small intercellular spaces, interpreted as dilated bile capillaries. These spaces contained serous precipitate, fibrin, and occasional leucocytes. Note the sharply outlined boundaries of the spaces and the manner in which these spaces ramify between cells. This rat was exposed by the feeding of large daily doses for 12 days. Similar but less marked changes resulted from the feeding of small doses over a period of 23 days (see fig. 2). After 75 days' exposure by inhalation to low concentrations of this preparation, marked liver changes, consisting of cellular swelling, vacuolization,

and hyalinization were apparent (fig. 2).

FIGS. 4, 5, AND 6. The microscopic changes resulting from exposure to chlorinated diphenyl are illustrated in these figures. Figure 4 was made from the liver of a rat fed large daily doses for only 6 days. Marked swelling of cells and rapid regenerative activity are apparent. Figure 5 illustrates the changes that were uniformly produced by the feeding of small doses of this preparation. Note the extremely numerous hyaline inclusions in liver cells. This rat was exposed for 29 days. Very similar changes were produced by the administration of this compound by inhalation methods (fig. 6). The rat from which this drawing was made had been exposed to low concentrations for a period of 107 days.

FIG. 1. Camera lucida drawing $\times 95$.

FIGS. 2 TO 6. Camera lucida drawings $\times 395$.

PLATE III



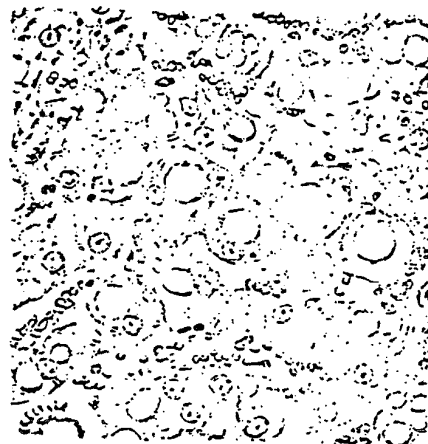
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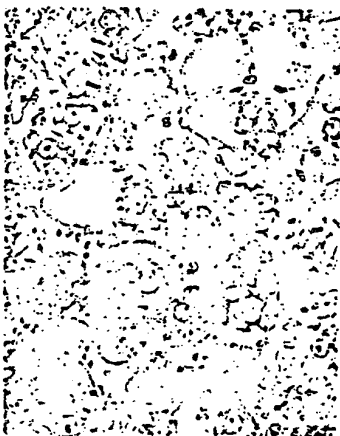
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amounts of fat. These changes increased slightly with longer exposure. (See plate II, figs. 1 and 2.) There were no architectural changes in the liver.

Because the effects resulting from large doses of trichloronaphthalenes were slight, this compound was not fed in small amounts.

Compound B. Tetra- and pentachloronaphthalenes

Exposure by feeding.—This preparation was employed only in feeding experiments. The daily food ration for 10 rats contained 0.5 gm. of this compound. All animals fell ill and either died or were sacrificed by the sixty-third day.

The livers were pale yellow in color, friable, and occasionally showed mottling. There were no significant variations in weights. On microscopic examination the same changes were observed in all rats. These consisted of moderate to marked swelling and rounding of the liver cells. The sinusoids were markedly narrowed. The majority of the liver cells contained large numbers of small fat vacuoles. This finding was most marked and first observed in the central portions of the lobules. In the least altered cells there was prominent massing of basophilic granules near the nuclei. Occasional necrotic cells were observed. Mitotic figures

were present in increased numbers, indicating accelerated regenerative activity. The microscopic changes resulting from the feeding of tetra- and pentachloronaphthalenes are illustrated in plate I, figures 3 and 4. They are those of diffuse fatty infiltration and fatty degeneration.

Compound C. Tetra- and Pentachloronaphthalenes plus Chlorinated Diphenyl

Exposure by feeding.—Feeding (3 gm. doses daily per 10 rats) was continued with but one 4 day interruption for 130 days. Ten rats were so-treated. Seven rats were sacrificed at varying periods of 47 to 124 days. Three rats died after exposure periods of 122 to 130 days. In all animals the livers were enlarged (33 to 90 per cent). The average weight increase was 71 per cent. They were also friable, pale yellow in color, and somewhat mottled. On microscopic examination, constant changes were observed. Practically every liver cell was swollen and rounded. Their cytoplasm contained large numbers of hyaline bodies. These were circular or oval in shape and varied in size from about half the size of a red blood corpuscle to twice the size of the nucleus of a liver cell. (See figs. 1 and 2, plate I.) In numerous instances, many small hyaline bodies had fused, forming large circular masses as large or larger than a normal

PLATE IV

FIG. 1. Photomicrograph $\times 45$ illustrating the extent of the liver damage produced in 48 hours by 0.75 cc. per kgm. each of carbon tetrachloride and ethyl alcohol when administered by stomach tube to a normal control rat. The degenerative changes were limited to the central portion of the lobules.

FIG. 2. Photomicrograph $\times 220$ of a

section of liver tissue from a rat exposed by inhalation methods to trichloronaphthalenes in low concentration for 150 days. Following this exposure 0.75 cc. per kgm. each of carbon tetrachloride and ethyl alcohol was administered by stomach tube. The animal survived and was sacrificed 17 days later. No significant liver changes are demonstrable.

PLATE IV

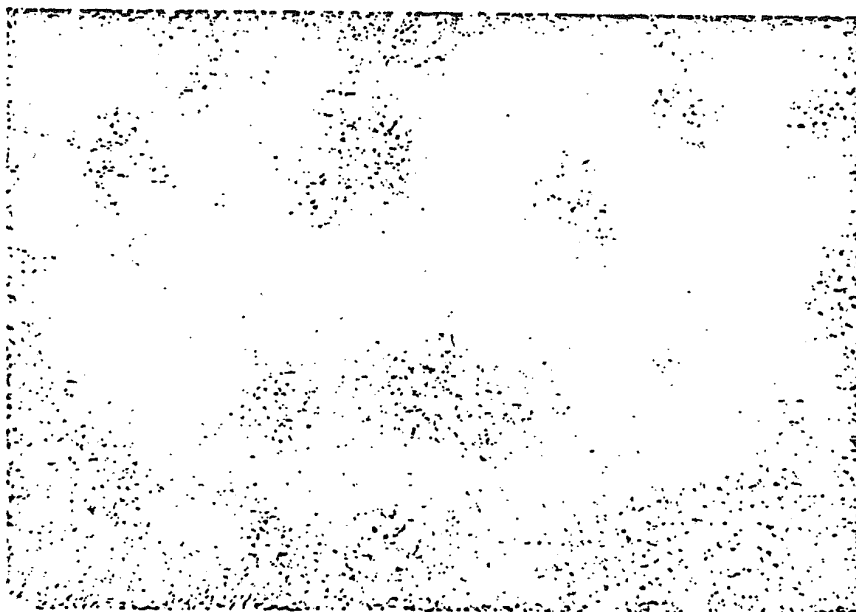


FIG. 1

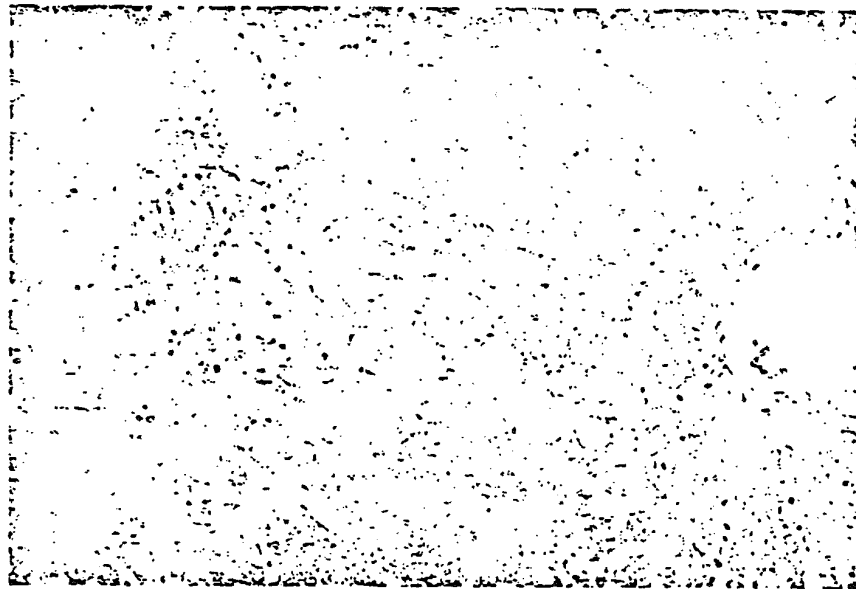


FIG. 2

liver cell. These bodies stained brilliantly with eosin dye. They were often laminated and occasionally contained small clear fat vacuoles in their central portions. Mitotic figures in liver cells were sufficiently numerous to indicate an increased rate of regeneration (fig. 1, plate I). In the livers of 2 rats exposed for 124 and 125 days respectively, there were, in addition to the above changes, large areas of complete liver cell degeneration (fig. 2, plate I). For the most part this was limited to the central half or third of the liver lobules. Occasionally, however, there was complete degeneration of all liver cells except for a narrow zone of cells around the portal areas. In such livers there was a heavy polymorphonuclear and mononuclear inflammatory cell infiltration in the necrotic areas where liver cells were being removed.

The most conspicuous feature of the microscopic changes in the livers of these rats was the presence of large numbers of circular hyaline droplets in the cytoplasm of the liver cells. This material did not stain in a manner characteristic of amyloid and its staining properties were not like those of the hyaline observed in alcoholic cirrhosis. In frozen sections stained with Scharlach R, the staining reac-

tion of these bodies was inconstant. The larger vacuolated hyaline bodies contained small droplets of fat while the majority of the smaller droplets did not stain red. Although similar hyaline droplets were observed in livers of rats exposed to various chlorinated naphthalenes, this type of degeneration occurred much earlier and to a much more marked degree in those rats that were exposed to preparations containing chlorinated diphenyl (figs. 1 and 2, plate I) or to chlorinated diphenyl alone (figs. 4, 5, and 6, plate III).

Compound D. Penta- and hexachloronaphthalenes

(a) *Exposure by inhalation to low concentrations (average 1.16 mgms. per cu. m.) 16 hours daily.*—Eighty rats living under these conditions appeared normal throughout the exposure period (134 days). Representative animals were sacrificed in groups of 3 to 15 animals after varying exposure periods as described in the experiments employing trichloronaphthalenes. There were no significant alterations in liver weights. Macroscopically the majority of the livers were light yellow in color and slightly mottled. After the initial exposure period of 37 days there was evidence of slight injury to

PLATE V

FIG. 1. A photomicrograph $\times 120$ showing small areas of necrosis in central areas of the liver lobules. This rat had been exposed by inhalation to low concentrations of trichloronaphthalenes for 134 days following which carbon tetrachloride and ethyl alcohol were administered by stomach tube in doses of 0.75 cc. each per kgm. The rat was sacrificed 5 days later. The extent of necrosis is similar to that resulting from carbon tetrachloride and alcohol alone.

FIG. 2. In this photomicrograph $\times 45$ are shown the most marked liver changes

that were observed following the administration of carbon tetrachloride and alcohol (0.75 cc. of each per kgm.) to rats which had been exposed by inhalation methods to high concentrations of trichloronaphthalenes. This rat had been exposed to trichloronaphthalenes for 1 month. Very extensive necrosis was present in the central areas of each liver lobule. In occasional areas the major portion of several adjacent lobules were necrotic. This rat was autopsied 48 hours after the administration of carbon tetrachloride.

PLATE V

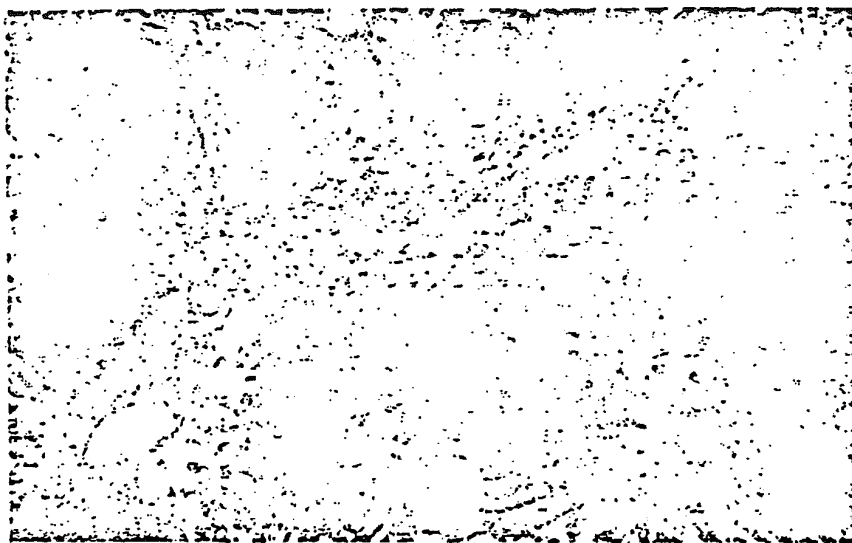


FIG. 1



FIG. 2

liver cells which appeared swollen and more granular than normal. The cytoplasm of occasional cells contained small acidophilic hyaline droplets and there was a moderate excess of fat in the form of tiny vacuoles (fig. 6, plate II). All microscopic abnormalities increased slightly during the second period (37 to 72 days) but no significant advance was detected between the 72nd and 134th day of exposure.

The livers of rats exposed for 105 days and then removed from exposure for a period of 2 months still showed cellular changes similar to those observed at the beginning of the recovery period. In a few of the specimens they appeared slightly less marked, indicating that some repair had taken place. In no instance was there evidence of increased damage.

Carbon tetrachloride and ethyl alcohol administered to rats exposed in the above manner for 144 days proved highly fatal, 9 out of 10 rats dying within the first 6 days—6 within 72 hours. At autopsy the livers of these rats were yellow and mottled and they were increased in weight (16 to 44 per cent). Microscopic examination showed widespread central necrosis similar to that observed in early acute yellow atrophy of man (see

figs. 1 and 2, plate VII). The central one-half or two-thirds of each liver lobule showed complete liver cell necrosis and degeneration. Diffuse extravasation of erythrocytes had occurred and numerous polymorphonuclear and mononuclear leucocytes had invaded the necrotic areas. In the narrow periportal zones where the liver cells were less markedly damaged, there were large numbers of mitotic figures (fig. 2, plate VII).

(b) *Exposure by inhalation to low concentrations (average 1.44 mgms. per cu. m.) 8 hours daily.*—Animals were subjected to these conditions for 143 days. Groups of animals sacrificed after 42, 77, 98, 119, and 143 days' exposure showed liver changes that were essentially like those seen in rats exposed 16 hours daily. The carbon tetrachloride and alcohol test was uniformly fatal and produced liver changes that were identical to those already described and illustrated (figs. 1 and 2, plate VII).

(c) *Exposure by inhalation to high concentrations (average 8.88 mgms. per cu. m.) 16 hours daily.*—Penta- and hexachloronaphthalenes when administered in this concentration proved highly toxic. Eighty animals were

PLATE VI

FIG. 1. Photomicrograph $\times 220$ of the liver of a rat exposed to penta- and hexachloronaphthalenes by the feeding of large daily doses for 16 days. The liver cells are swollen and markedly vacuolated. There is distortion of the liver cell columns. In frozen section preparations stained with Scharlach R, there was evidence of marked fatty degeneration. In addition to these changes, numerous oval, round and irregular intercellular spaces are present throughout the liver lobules. Such spaces contain a granular serous precipitate, strands of

fibrin and occasional leucocytes. These spaces, insofar as could be determined, were due to dilatation of the bile capillaries and canaliculi.

FIG. 2. This photomicrograph $\times 215$ when compared with the above figure demonstrates the similarity of the liver changes produced by the same compound administered in a different manner. In this instance the rat inhaled high concentrations of penta- and hexachloronaphthalenes for 38 days.

PLATE VI

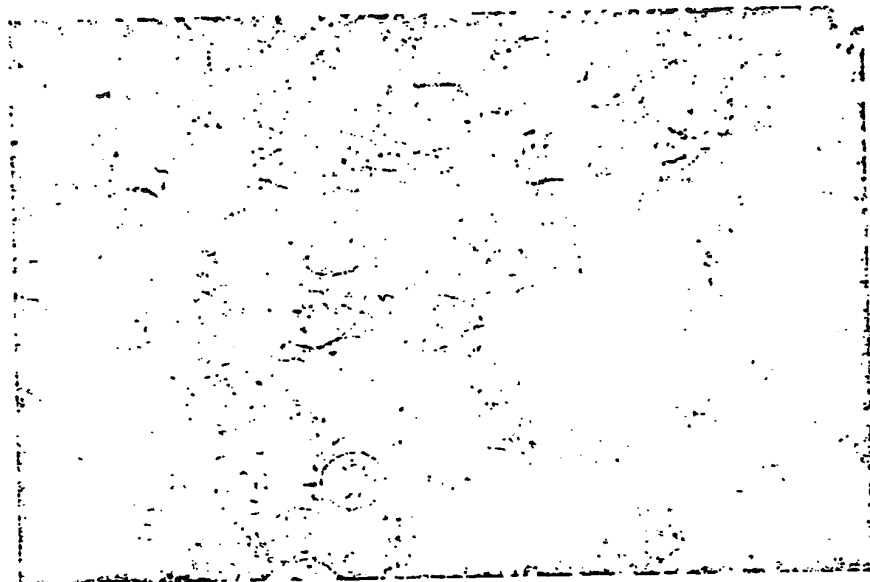


FIG. 1

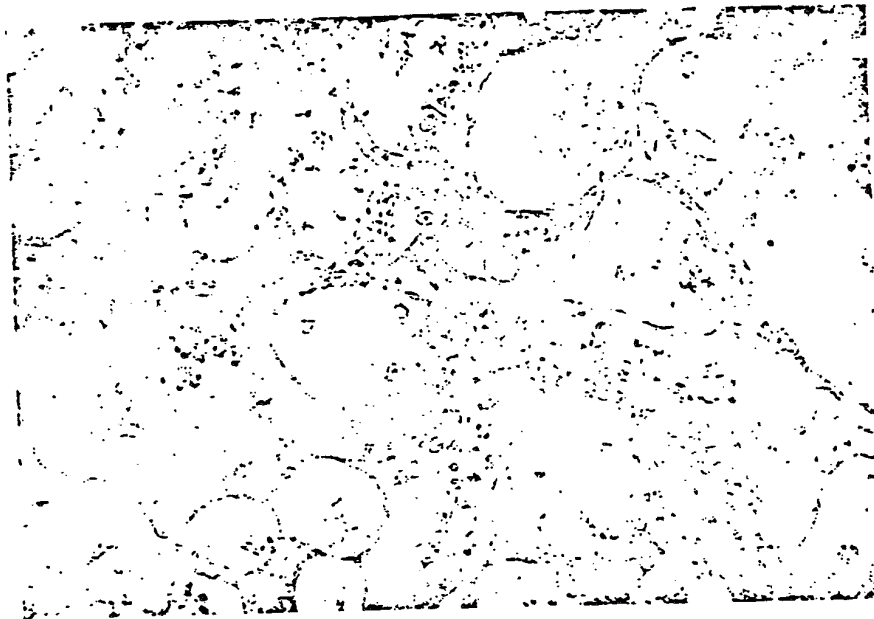


FIG. 2

used, all of which lost weight and appetite. Four died by the end of the first month, 55 within 52 days. Most of these were markedly jaundiced. The remainder were sacrificed for pathological examination or succumbed to the effects of carbon tetrachloride and alcohol. Only 8 animals survived the 52 day exposure. The majority of livers were moderately or markedly enlarged. They were all yellow and in addition the majority were markedly mottled. Occasionally they were granular and their cut surfaces showed minute spaces which became more prominent after fixation. After an 8 day exposure, the liver cells were markedly swollen and moderate fatty degeneration was present. This was most marked in the central areas although all portions of the liver lobules were affected. The extent of liver injury rapidly increased so that after 30 days' exposure, marked structural as well as cellular changes were apparent. The microscopic appearances were identical to those to be described in rats fed large doses of penta- and hexachloronaphthalenes (figs. 1 and 2, plate VI) and in rats fed similar doses of 90 per cent penta- and hexachloronaphthalenes plus 10 per cent chlorinated diphenyl (see fig. 1, plate III and figs. 1 and 2, plate VIII).

Rats removed from exposure between the third and fifth week continued to die, the longest survival time being 35 days. Microscopic examination of the livers of these animals revealed no evidence of recovery.

Administration of carbon tetrachloride and alcohol again proved fatal to animals exposed in the above manner. The livers of such animals showed the characteristic massive necrosis previously described and illustrated.

(d) *Exposure by feeding.*—When fed in doses of 3 gm. daily to a group of 10 rats, penta- and hexachloronaphthalenes produced marked cellular and structural changes in the liver in a short period of time. All rats lost weight and appeared ill from the beginning. The longest survival time was 33 days. Although the liver edges were blunt and rounded, there were no significant weight variations. The livers were friable, yellow and mottled. Microscopically they showed marked swelling and vacuolization of the cells. There was also complete degeneration of scattered cells. Occasional mitotic figures were observed (see fig. 4, plate II). Suitable stains revealed very marked fatty degeneration. Occasional cells contained oval shaped or circular acid-

PLATE VII

Fig. 1. A low power photomicrograph $\times 45$ illustrating the marked degree of degeneration of liver tissue resulting from the administration of a small dose of carbon tetrachloride and ethyl alcohol (0.75 cc. of each per kgm.) to an animal that had been exposed previously by inhalation methods to low concentrations of penta- and hexachloronaphthalenes for 134 days. This animal died within 24 hours after the administration of carbon tetrachloride.

Similar degrees of necrosis resulted from the administration of carbon tetrachloride and alcohol to rats which had been exposed to a mixture of 90% penta- and hexachloronaphthalenes and 10% chlorinated diphenyl or to refined chlorinated diphenyl.

Fig. 2. A higher power photomicrograph $\times 225$ of the liver illustrated in the above figure.

PLATE VII



FIG. 1

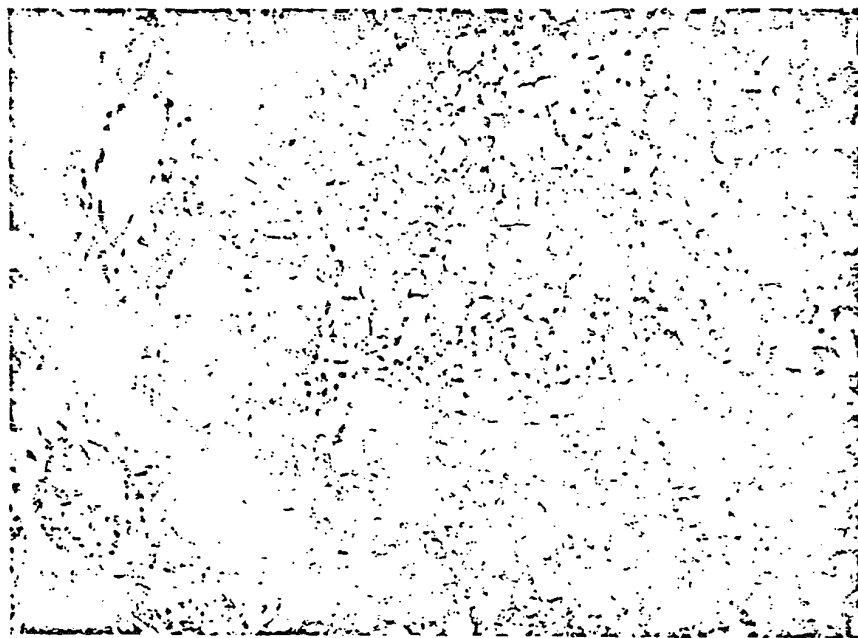


FIG. 2

ophilic hyaline inclusions. In addition to these cytological changes there were numerous varying sized intercellular spaces throughout the livers (see figs. 1 and 2, plate VI and fig. 1, plate III). These spaces were usually circular or oval in shape, although many of them ramified between cells in the liver columns or between liver cells and the sinusoidal endothelium in such a way as to suggest that they represented greatly dilated bile capillaries or canaliculi (fig. 1, plate III). They contained serous precipitate, a few strands of fibrin, and occasionally a few leucocytes. Rarely one observed one or two degenerating liver cells within these spaces. In some of the more markedly damaged livers, red blood corpuscles were also present. The above microscopic changes appeared identical to those observed in the livers of rats subjected to high concentrations of this compound by inhalation methods (figs. 1 and 2, plate VI). They were also similar to the changes that resulted from the feeding of another penta- and hexachloronaphthalene compound (figs. 5 and 6, plate I) and from the feeding of a mixture of penta- and hexachloronaphthalene (90 per cent) and chlorinated diphenyl (10 per cent).

When this preparation of penta- and hexachloronaphthalenes was fed in smaller amounts (0.5 gm. every second

day to a group of 4 rats) the above described liver changes occurred less rapidly and to a lesser degree (see fig. 5, plate II).

Compound E. Penta- and Hexachloronaphthalenes

Exposure by feeding.—Feeding in doses of 1 gm. daily for 10 rats was continued with but one 4 day interruption. All 10 rats had died or had been sacrificed by the fifty-fifth day. These livers were friable and yellowish, and exhibited moderate to marked mottling. One animal was jaundiced. Of the 5 animals that were sacrificed, three showed enlargement of the liver (30 to 40 per cent); the remaining livers were normal in size. On microscopic examination the livers of rats exposed for 2 weeks showed marked cellular changes that appeared to be degenerative in nature. Although the liver cell injury was somewhat greater in the central portions of the liver lobules, no portion of the liver was spared. The liver cells showed varying degrees of swelling, increased granularity and vacuolization of the cytoplasm. Early injury was indicated by a massing of basophilic granules and small rod-shaped structures near the cell nuclei. The peripheral portions of such cells were acidophilic and vacuolated. Small irregular spaces were present between

PLATE VIII

FIG. 1. A photomicrograph $\times 95$ of the liver of a rat fed a mixture of 90% penta- and hexachloronaphthalenes and 10% chlorinated diphenyl, in 3 gm. doses per 10 rats for 12 days. This rat survived for 23 days after feeding was stopped. A comparison of the liver of this rat with those of rats sacrificed earlier indicated that the liver changes had progressed considerably after administration of the compound had been terminated.

One should note the marked change in the liver architecture. The spaces like those previously illustrated (figs. 1 and 2, plate VI) are very large and the remaining liver tissue is compressed. The liver cells show marked fatty degeneration.

FIG. 2. A higher power photomicrograph $\times 225$ from another area in the liver illustrated in the above figure.

PLATE VIII

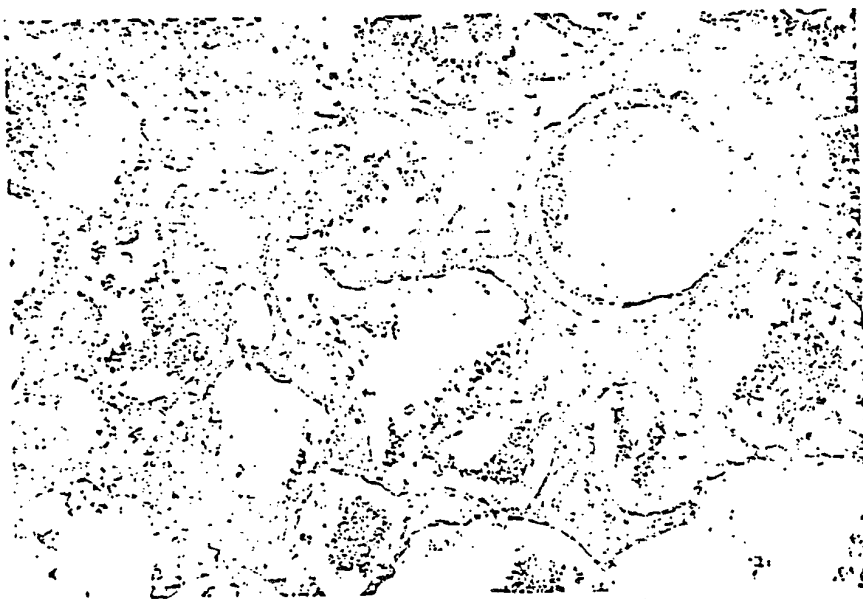


FIG. 1

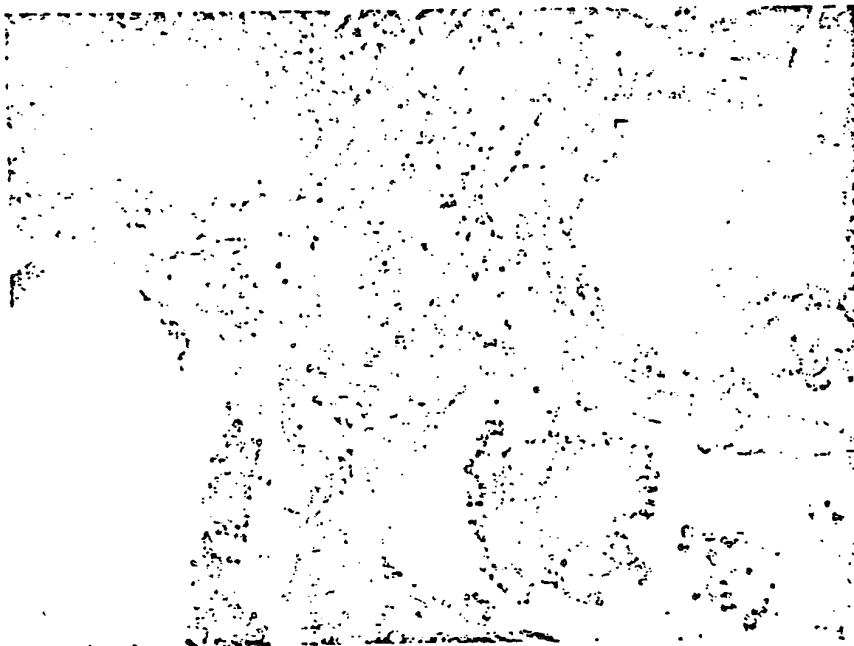


FIG. 2

liver cells and between the columns of liver cells and the sinusoidal endothelium (see fig. 5, plate I). These were interpreted to be the early stages in the formation of large intercellular spaces (fig. 6, plate I) which subsequently developed and which were identical to the spaces described in the foregoing section. After longer exposure the liver cells showed very marked swelling and fatty infiltration. Occasional degenerating and regenerating cells were observed.

Compound F. 90 per cent Penta- and Hexachloronaphthalenes and 10 per cent Chlorinated Diphenyl

(a) *Exposure by inhalation.*—Inhalation experiments were carried out on two groups of rats. The first of these groups of 80 animals were exposed to low concentrations (average 1.37 mgms. per cu. m.) 16 hours daily for 134 days. The second group, also comprised of 80 rats, was exposed to low concentrations (average 1.66 mgms. per cu. m.) 8 hours daily for 143 days. No evidence of illness was noted in the living animals in either group. In each experiment representative groups of animals were sacrificed after exposure periods corresponding to those used in low concentration inhalation experiments with trichloronaphthalenes and penta- and hexachloronaphthalenes.

Since there were no recognizable differences in the amount or type of liver change observed after similar exposure periods in these two groups of rats, the second group (exposed 8 hours daily) will not be discussed.

Macroscopically the livers were usually light yellow. After prolonged exposure, this abnormality was

more marked and in many instances mottling was present. There were no very significant alterations in the weights of livers although the majority were slightly swollen (average of 20 per cent increase in weight). Microscopically the livers showed constant changes after 37 days of exposure. These increased somewhat during the second period of 35 days. Longer exposure produced little if any increase in liver damage. The observed pathological changes consisted of swelling and rounding of liver cells accompanied by a definite increase in the prominence of the cytoplasmic granules. Hyaline droplets in the altered cytoplasm were a conspicuous feature (see fig. 3, plate III). Mitotic figures were present in abnormally large numbers. In an occasional liver, as was the case with similar exposure to both penta- and hexachloronaphthalenes alone and refined chlorinated diphenyl, there was a marked deposit of finely divided granular and refractile yellow or yellow brown pigment. This was present within liver and Kupffer cells. Larger amounts were found in the central portions of the liver lobules. It failed to stain in a manner characteristic of either hemosiderin or hemofuscin.

The livers of rats removed from exposure for 2 months after an exposure period of 105 days were similar to those of animals sacrificed at the end of 105 days' exposure. There was, however, no evidence of any increase in the pathological changes.

The administration of carbon tetrachloride and alcohol was almost uniformly fatal. Macroscopic examination of livers of these rats revealed marked enlargement (approximately

50 per cent). Such livers were yellow and mottled. Again there was extensive and widespread liver necrosis on microscopic examination (see figs. 1 and 2, plate VII).

(b) *Exposure by feeding.*—When fed in large doses (3 gm. daily for 10 rats) this compound proved to be very toxic. All animals appeared ill and feeding of the compound was stopped after 12 days. Despite this the rats continued to die, the last dying on the 35th day. Subsequent microscopic examination revealed that the liver changes had continued to progress after feeding was stopped. Macroscopically, the majority of the livers were enlarged, the largest showing an increase in weight of 118 per cent; the average increase was approximately 40 per cent. All livers were yellow, friable, and many of them were markedly mottled. Subsequent microscopic examination indicated that much of the mottling was due to hemorrhage into spaces within the liver tissue. In addition, the external and cut surfaces of the livers appeared slightly pitted or granular. The microscopic changes observed in the livers of the animals autopsied after 10 to 14 days' exposure consisted of moderate to marked swelling of liver cells accompanied by marked fatty vacuolization. Occasional liver cells showed additional signs of injury such as nuclear degeneration and polymorphonuclear leucocytic invasion. Scattered circular or oval shaped intercellular spaces (fig. 1, plate III) like those previously described in experiments where penta- and hexachloronaphthalenes were employed (figs. 1 and 2, plate VI) were observed. In

the less markedly damaged livers, such spaces contained serous precipitate, strands of fibrin, and small numbers of leucocytes. In rats surviving 18 to 35 days, these spaces were much larger and often had attained the size of a normal liver lobule (see figs. 1 and 2, plate VIII). In these more severely damaged specimens, extensive hemorrhage had occurred (fig. 2, plate VIII). In sections stained with P.T.A.H. these spaces were seen to be bounded by flattened liver cells which showed sharp cell borders. Liver cells between such spaces were distorted, swollen, and showed marked fatty degeneration. In many of these livers there was a slight increase in connective tissue between the remaining cords of liver cells and around recognizable portal areas. Proliferative changes were occasionally observed in the bile ducts. This was indicated by increased numbers of ducts in certain areas and by mitotic figures in the bile duct epithelial cells. No recognizable dilatation of medium sized or large bile ducts was present. In many sections the architecture of the liver was so completely altered that it was difficult to recognize the portal areas. In all sections there was evidence of fatty and hyaline degeneration of the remaining liver cells. Slight polymorphonuclear leucocytic infiltration was present. This was somewhat more marked in the periportal areas.

Feeding of this mixture of penta- and hexachloronaphthalenes and chlorinated diphenyl in smaller doses (0.5 gm. every second day for 4 rats) resulted in liver damage that developed less rapidly. The rats were sacrificed after exposure periods of

21, 34, 58, and 81 days. Their livers were yellow, increased in size and showed slight mottling. Microscopically, the chief abnormality observed was marked fatty vacuolization of liver cells. No intercellular spaces like those described above were present.

Compound G. Chlorinated Diphenyl

(a) *Exposure by inhalation.*—Exposure by inhalation methods to low concentrations of chlorinated diphenyl was carried out on two groups of animals, each comprised of 80 rats, in a manner identical to that described in the foregoing sections. In the first group a 16 hour daily exposure, with an average concentration of 0.57 mgms. per cu. m., was employed. The second group of animals was exposed 8 hours daily to an average concentration of 0.93 mgms. per cu. m. Neither group of rats appeared ill. Representative animals were sacrificed in groups of 3 to 21 rats, after varying periods of exposure. In the first experiment, the animals were sacrificed after 37, 72, 105, and 134 days of exposure, in the second experiment after 42, 77, 98, 119, and 143 days. The livers were pale or slightly yellow and somewhat mottled. There were no constant variations in the weights of the livers. The microscopic findings in the two groups indicated that the different exposures had resulted in the same degree of liver injury. The pigmentation of the liver and Kupffer cells was somewhat greater in animals exposed 8 hours daily. In both experimental groups the carbon tetrachloride and alcohol test was highly fatal and led to the same extensive central necrosis of liver tissue that was observed in rats exposed to

the toxic chlorinated naphthalene compounds (figs. 1 and 2, plate VII). The microscopic changes resulting from exposure to low concentration of chlorinated diphenyl alone were similar to those resulting from inhalation of penta- and hexachloronaphthalenes, plus 10 per cent chlorinated diphenyl. The most conspicuous change was hyaline degeneration (fig. 6, plate III) although swelling of liver cells, increased prominence of cytoplasmic granules and increased vacuolization were present. Mitotic figures were present in increased numbers. No microscopic evidence of recovery was found in the livers of rats exposed for 105 days and then removed from exposure for a period of 2 months.

(b) *Exposure by feeding.*—When fed in large daily doses (3 gm. for groups of 10 rats), chlorinated diphenyl proved highly toxic. Feeding was discontinued after 6 days. Seven rats died within the first 8 days. The 3 remaining rats gained in weight after the feeding was stopped and were finally sacrificed. The last animal was sacrificed 63 days after feeding was begun. The majority of livers showed a moderate increase in weight. Microscopically there was evidence that liver injury had occurred within the first few days. The cells were swollen, at times sufficiently to obscure the sinusoids. The cytoplasm of the liver cells was acidophilic in staining quality and contained small vacuoles. Small eosin-stained hyaline granules and globules were present. Mitotic figures were numerous (see fig. 4, plate III). There was little inflammatory cell infiltration and no structural changes were observed.

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When smaller doses (0.5 gm. every second day) of this compound were administered to 10 rats, the first death occurred after 9 days. Two additional deaths occurred after 12 days and two more by the end of the fifth week. The remaining animals were sacrificed between the 40th and the 190th day. The livers were enlarged, the average increase in liver weight being 33 per cent. They were pale and slightly mottled. The most outstanding microscopic abnormality was the presence of large numbers of varying sized acidophilic hyaline globules in the cytoplasm (see fig. 5, plate III). These hyaline globules, although larger and more numerous, appeared identical to those encountered in the livers of rats exposed to other chlorinated diphenyl preparations. The liver cells were markedly swollen and showed fatty vacuolization. There was also increased regenerative activity. No structural changes were observed in any of the livers.

DISCUSSION

Published reports concerned with the possible systemic effects of chlorinated naphthalenes have been few in numbers. Lehmann (3) in 1919 reported that animals exposed to chlorinated naphthalenes by inhalation or feeding refused to eat and at death showed "peculiar" lesions in the liver. In 1936, Flinn and Jarvik (4) reported the results observed following the daily subcutaneous injections into 30 rabbits of enormous doses (30 mgm.) of certain chlorinated naphthalene compounds dissolved in paraffin oil. The rabbits receiving a mixture of tri- and tetrachloronaphthalenes lived and when sacrificed, after a 2 months'

exposure, no pathological changes attributable to the compound injected were demonstrable. The mixtures of tetra- and pentachloronaphthalenes and of penta- and hexachloronaphthalenes proved highly fatal and degeneration of liver tissue was observed. From their findings Flinn and Jarvik concluded that certain chlorinated naphthalenes or impurities contained in them are capable of producing yellow atrophy of the liver in the rabbit. It is noteworthy, in the light of the present experiments, that the compound of lowest chlorination (a mixture of tri- and tetrachloronaphthalenes) had no apparent effect on the well being of the animals and did not produce demonstrable changes in the liver although injected daily for a period of 2 months.

The present experiments demonstrate that chlorinated naphthalene compounds and chlorinated diphenyl are capable of producing marked liver damage in the white rat without demonstrable microscopic changes appearing in the other organs. Furthermore, the characteristics of the liver lesions resulting from comparable amounts of any given compound are the same, regardless of the method of administration (inhalation, feeding, or subcutaneous injection).

An attempt has been made throughout this study to grade the toxicity of each of the several compounds tested in accordance with the severity of the liver damage produced. Although some of these compounds appeared to produce similar degrees of liver injury, the toxicity of each compound seemed directly related to its degree of chlorination. Thus, a mixture of trichloronaphthalenes (chlorine content 49.4

per cent) proved to be the least toxic of any of the compounds tested, whereas chlorinated diphenyl (chlorine content 65.0 per cent) was highly toxic even in very low concentrations.

Trichloronaphthalenes, when compared to other naphthalene compounds of higher degrees of chlorination, were relatively innocuous. Even after prolonged feeding of large amounts of this material, the rats showed no evidence of ill health. After 2 months' exposure, microscopic examination of the livers revealed only slight to moderate degrees of fatty infiltration and degeneration of liver cells (plate II, figs. 1 and 2). These changes were slight when compared to those resulting from short exposures to compounds of higher chlorination. Exposure by inhalation to low concentrations of trichloronaphthalenes produced liver cell alterations that were never more than minimal (see fig. 3, plate II). Such animals withstood additional liver injury from carbon tetrachloride and alcohol in a manner indistinguishable from that of the normal animal. Somewhat greater liver injury resulted from inhalation when the air concentration of this compound was increased more than eight times that used in the previous experiment but again the liver changes were slight compared to those produced by comparable exposure to the more highly chlorinated compounds.

The mixture of tetra- and pentachloronaphthalenes employed in these experiments appeared to be considerably more toxic than was the mixture of trichloronaphthalenes. Animals fed small doses of the former of these preparations fell ill and died or were sacrificed by the 63rd day. Their livers showed extensive fatty infiltra-

tion and fatty degeneration (figs. 3 and 4, plate I).

Feeding of tetra- and pentachloronaphthalenes in combination with chlorinated diphenyl resulted in pronounced liver changes. These livers had increased in weight (average 71 per cent). Microscopic examination revealed a peculiar type of hyaline degeneration involving practically every liver cell (see figs. 1 and 2, plate I). This type of cell degeneration was more marked and occurred earlier after exposure to preparations containing chlorinated diphenyl than to any other compounds tested. Furthermore, it was most marked in the livers of animals exposed to refined chlorinated diphenyl (figs. 4, 5, and 6, plate III).

Comparable exposure of rats to penta- and hexachloronaphthalenes (Compound D), penta- and hexachloronaphthalenes (Compound E), and penta- and hexachloronaphthalenes (90 per cent) plus chlorinated diphenyl (10 per cent) (Compound F), resulted in liver changes having few detectable differences.

The morphological changes were slightly greater in the livers of rats exposed to the last named compound. In these instances the hyaline degeneration of the cell cytoplasm was more marked. Although rats inhaling low concentrations of compounds D and F showed no demonstrable signs of ill health, microscopic examination of their livers revealed marked liver cell injury (fig. 6, plate II, and fig. 3, plate III). These lesions were still demonstrable after a 2 months' recovery period. Further evidence that liver damage had resulted from inhalation in low concentrations of these compounds was obtained from the carbon tetra-

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chloride and alcohol test. Such tests were almost uniformly fatal and resulted in widespread liver necrosis (figs. 1 and 2, plate VII). These three compounds proved to be very toxic and resulted in a most unusual type of liver change when administered in large amounts by feeding (see fig. 1, plate III, fig. 1, plate VI, and figs. 1 and 2, plate VIII). Identical changes were observed following inhalation in high concentrations of compound D (see fig. 2, plate VI). In such livers the most conspicuous feature was the presence of large intercellular spaces. Examination of routine sections, supplemented by specially stained preparations indicated that these spaces had resulted from dilatation of bile canaliculi. The reason for such dilatation was not apparent. There was no corresponding dilatation of the large and small bile ducts at the periphery of the liver lobules and no inspissated bile was ever observed. The organically combined chloride in the livers of these rats was no greater than that observed in the normal animal. Although serious liver damage results from exposure to high concentrations of these compounds, the resulting changes are most unusual and do not resemble those of acute yellow atrophy on microscopic examination.

Of the various chlorinated hydrocarbons tested, chlorinated diphenyl gave evidence of being the most toxic. When administered by inhalation in very low concentrations (average 0.57 to 0.93 mgms. per cu. m.) liver cell changes were very pronounced after the first exposure period. The most striking change was the hyalinization of the cell cytoplasm (see fig. 6, plate III). Such cellular alterations were

essentially unchanged after a 2 month recovery period. In these animals small sublethal doses of carbon tetrachloride and alcohol uniformly produced extensive liver necrosis and was highly fatal to them (figs. 1 and 2, plate VII). Chlorinated diphenyl fed in small doses produced similar but more marked liver injury (see fig. 5, plate III). In large doses this compound was highly fatal. The liver changes in animals dying after short exposures were inconspicuous and consisted mainly of swelling of cells and active regeneration (see fig. 4, plate III). However, animals removed from exposure before being fatally poisoned, subsequently developed hyaline degeneration of liver cells similar to that produced by prolonged administration of small doses of this compound.

Thus the results of the present study, as well as certain field studies that have been made (1) suggest that the solution of the industrial hazard involved is dependent largely on a reduction of the air concentration of these compounds to a level that will not produce liver damage. The present experiments indicate that lower air concentrations must be obtained in the case of the more highly chlorinated naphthalene compounds and chlorinated diphenyl than for trichloronaphthalenes if a safe environment for workmen is to be assured. Because of the pronounced toxic effect of small doses of carbon tetrachloride on the livers of animals already injured by exposure to chlorinated naphthalenes and chlorinated diphenyl, its use as a solvent for these compounds would appear to be very hazardous.

Although this investigation was designed to determine what systemic

effects, if any, would be produced in experimental animals by a group of chlorinated hydrocarbons, the pathological changes produced have been of such a nature as to justify further comment. Exposure of rats to highly chlorinated naphthalene compounds and chlorinated diphenyl results in a type of liver injury differing considerably from the liver lesions resulting from carbon tetrachloride (5, 6) and chloroform poisoning (7). The changes resulting from exposure to the compounds employed in this study involve all portions of the liver lobule. This is true despite the fact that the initial morphological changes are more pronounced in the liver cells nearest the hepatic veins. The changes observed are degenerative in nature. The successive degenerative changes were cloudy swelling, fatty infiltration and fatty degeneration and finally, the complete disintegration of the cell. Degeneration characterized by the accumulations of large amounts of acidophilic hyaline material was also observed. This feature was much more marked in the livers of animals exposed to the compounds containing chlorinated diphenyl. It is of interest that widespread degenerative changes of all liver cells can exist for several months without producing any evidence of ill health in the animals or without causing important structural changes in their livers. Such findings suggest that the alterations take place slowly and that the scattered necrotic cells are efficiently removed and replaced by new cells which also become injured. There was no observable morphological evidence to indicate that the regenerating liver cells had acquired an increased resistance to the injurious effects of the compound

being used (8). The fact that liver cell changes were still present after a 2 month period during which the animals were removed from exposure to the more highly chlorinated compounds is further evidence that this type of injury is persistent and only slowly recovered from.

The administration in high concentrations of two compounds of intermediate degrees of chlorination resulted in marked structural changes in addition to marked degenerative changes in liver cells. The most conspicuous change was the occurrence of progressively enlarging intercellular spaces. These were interpreted as markedly dilated bile canaliculi. We are unaware of such changes having been previously described.

From the above it is apparent that exposure to highly chlorinated naphthalenes and chlorinated diphenyl not only results in liver changes having marked differences from those caused by other well known toxic agents, but also that the characteristics of the liver changes produced can be markedly altered quantitatively and qualitatively by varying the degree of exposure. For these reasons one would seem justified in suggesting that detailed studies on animals in which the types of liver injury observed in these experiments had been produced might further our understanding of liver cell function.

SUMMARY

1. Studies concerning the effect upon white rats of a group of chlorinated naphthalene compounds and chlorinated diphenyl are reported. This investigation was undertaken because of the recent occurrence of 3 fatal cases of jaundice in men work-

ing in industrial plants where these compounds were being used. The compounds tested were selected as representative of a certain range of chlorination and because of their relative industrial importance.

2. Four of the compounds tested were administered by inhalation. The air concentration and exposure periods were varied. All compounds were tested by feeding in large and small amounts. In addition three of them were tested on small numbers of animals by subcutaneous injections. The effects of any given compound did not appear to be influenced by the method of administration. However, marked quantitative and qualitative differences in the effects of each of several compounds resulted from varying the air concentrations in inhalation experiments and the dosage employed in feeding experiments.

3. Macroscopic and microscopic examination of the tissues of rats exposed to these compounds indicated that the injurious effects are manifested solely in the liver. Blood examination during life revealed no significant abnormalities.

4. The liver changes resulting from exposure to the chlorinated hydrocarbons employed have been described and illustrated. As indicated by their ability to injure liver tissue the toxicity of these compounds increases with increasing degrees of chlorination. Thus a mixture of trichloronaphthalenes is relatively innocuous in low concentrations and when compared with compounds of higher chlorination, its toxic properties even in higher concentrations are relatively slight. Chlorinated diphenyl appears to be the most injurious compound of all those tested.

5. Administration of small sublethal doses of carbon tetrachloride and ethyl alcohol to rats whose livers have already been injured by the compounds under consideration is highly fatal and produces massive necrosis of the liver.

6. The significance of this study in relation to the industrial problem involved is discussed.

7. It is suggested that the types of liver injury observed in this study might be employed in experiments designed to study liver cell function.

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Archival

REPORT TO THE MONSANTO CHEMICAL COMPANY
Cecil K. Drinker, M.D.
September 15, 1938

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PERGAD-Bayonne, N. J.
DEPOSITION
EXHIBIT
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2-15-94

NEV 130808

June 1937

REPORT ON 4465

C-25AM

Inhalation Experiments

Animals. White rats have been employed throughout. They permit the use of a large number of animals in a relatively small inhalation installation. They thrive upon a diet very similar to man, and in the case of these chlorinated compounds it is possible that diet may be very significant. Finally, their normal characteristics have been described so well as to make the detection of abnormalities both easy and certain.

Method of Exposure. The inhalation experiments were carried out in four large air-tight wooden boxes, each capable of holding ten rat cages, size 22" x 22" x 14", in two tiers of five cages each. When the experiment was not in progress the doors were opened wide and the cages kept in place (see Figure 1).

At the center of one end of each box -- the inflow end -- air was introduced through a pipe 7 inches in diameter (see Figure 2). Each box was equipped with an individual electric blower which blew the air through several feet of 7-inch pipe before entering the end of the box. An orifice was placed in the pipe line, and the flow of air in cubic feet per minute could be read off directly from calibrated flow meters. A damper placed at the entrance of the pipe into the box was adjusted to assure a uniform distribution of the stream of air to the two tiers of cages.

At the opposite end of the box -- the outflow end -- the air from each box was exhausted through a 7-inch pipe fitted with a damper and connected to a large central exhaust fan (see Figure 3).

About 4 inches from the entrance of the 7-inch pipe into the box, the fumes of the substance were introduced into the inflowing air.

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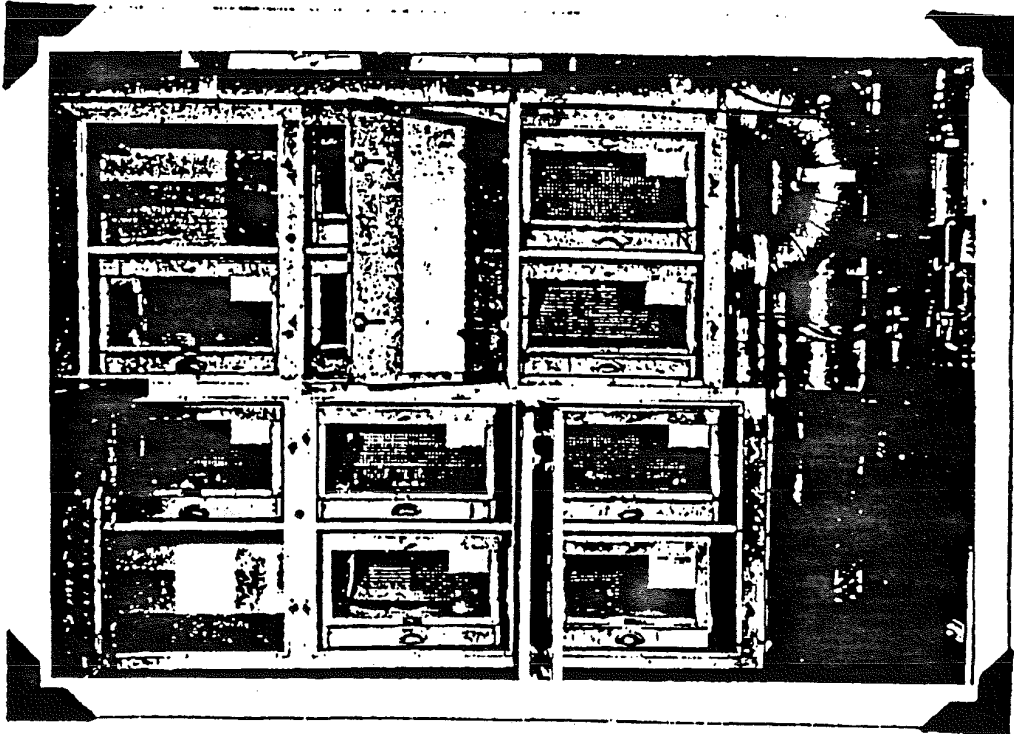


Fig. 1. Front view and inflow end of two boxes with rat cages in place and doors open.

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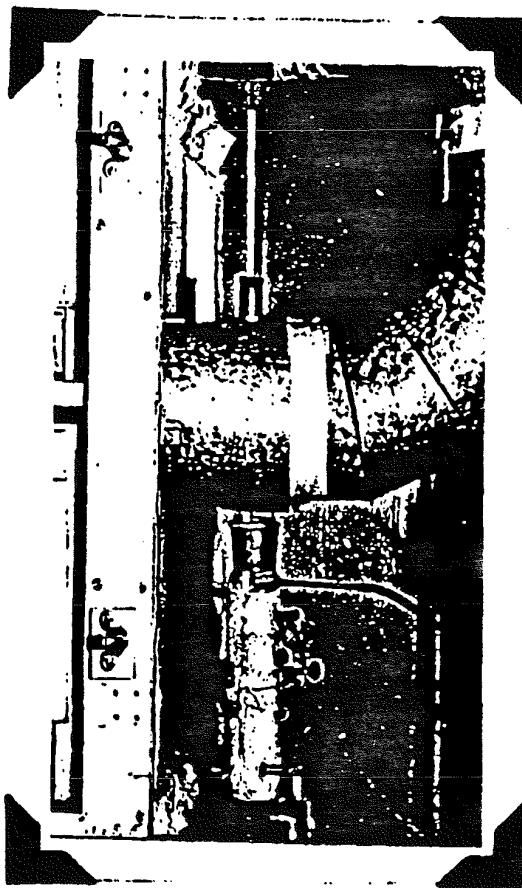


Fig. 2. Electric heater for maintaining chlorinated diphenyl at a constant temperature in place at inflow end of box.

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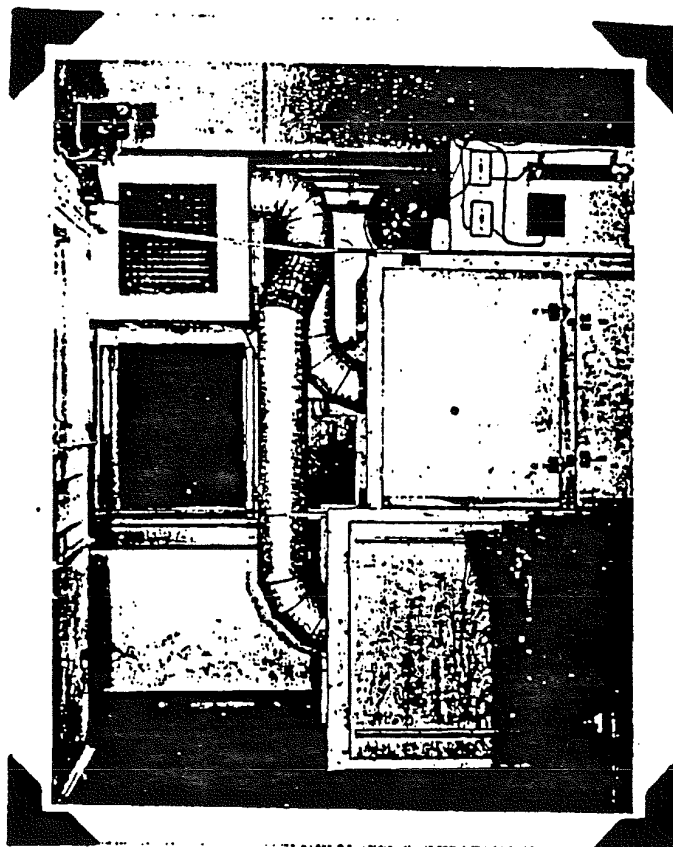


Fig. 3. Outflow end of two boxes showing connections to exhaust system.

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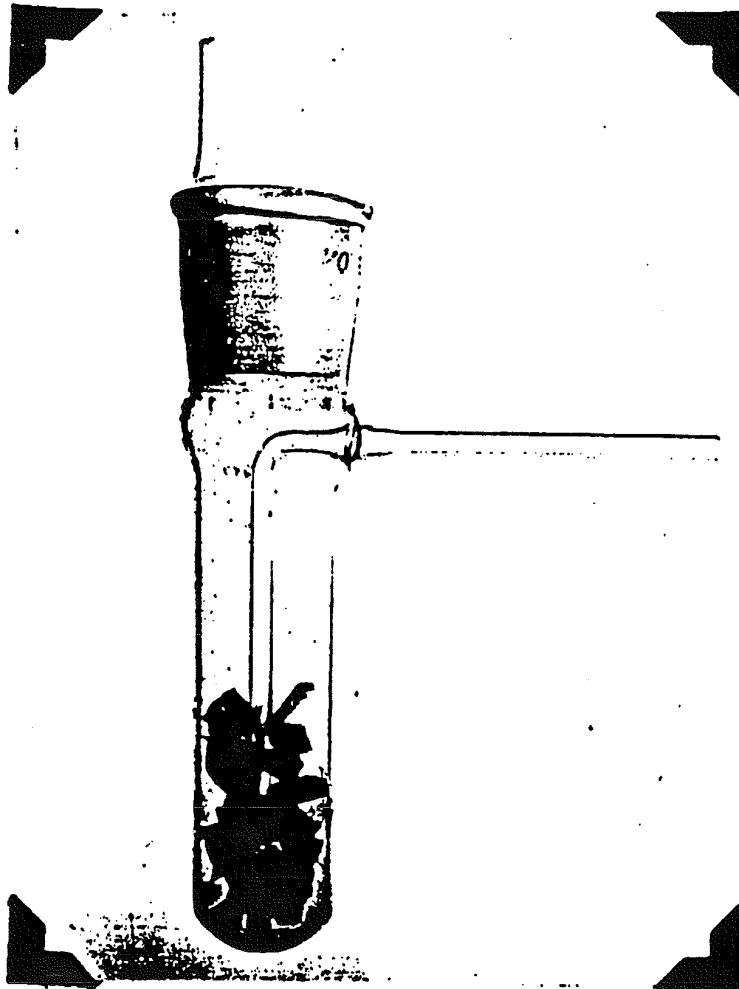


Fig. 4. Pyrex glass flasks.

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Specially designed pyrex glass flasks (Figure 4), 7 3/4" long and with a diameter of 1 1/2", were used to hold the heated waxes. These flasks were made with a side arm and tube which extended to the bottom. The top of the flask was ground, and into this fitted a short tube 1 1/2" in length. This short tube was inserted into a large rubber stopper which fitted tightly into a hole cut out of the 7-inch pipe on the under side (see Figure 2). The flask in turn was placed in an electric heater made to cover it completely below the side arm and ground glass top. Rubber tubing connected the side arm with a compressed air reservoir and a gentle stream of air blown through the melted wax kept it in motion and assured uniform heating. Into each flask was inserted a long stem centigrade thermometer which was kept in place and could be read at any time above the 7-inch pipe through which it passed (see Figure 2).

Approximately 20 gm. of pulverized ~~chlorinated diphenyl~~ ~~20 gm. of~~ chlorinated diphenyl were placed in the vaporizing flask and melted in the electric heater. Fresh samples were used every other day, ~~but it was often found necessary to add 20 gm. of new material when the collected sublimate was always removed from the upper part of the flask and a clean top used each day. No sample was ever used for more than two runs.~~

The flask plus the contents was carefully weighed at the beginning of the run and at the end, and the loss in weight used to calculate the average amount in a cubic meter of air per minute as determined by a series of flow meter readings. The figures obtained were not absolute because of slight variations in the air flowing through the boxes and because of deposition of material on the thermometers and on the inside surfaces of the box, but they checked well with direct determinations through air samples.

NEV 130814

Approximately one hour was allowed for the wax in the flasks to melt and come to a constant temperature. At that time the box doors were tightly closed, air bubbled through the flasks, and the blowers turned on. This was the beginning of the exposure period. By means of the theostats on the fans and the dampers in the outflow pipes, the amount of air flowing through the boxes was adjusted and an attempt was made to keep the four boxes as uniform as possible -- usually between 165 and 175 cubic feet per minute..

Experiments

Exposure to chlorinated diphenyl was begun on July 1, 1936 and ceased on November 18 of the same year. The average length of exposure was 16 hours daily for 6 days a week. Each morning at about 9:00 exposure ceased, and between this time and 4:00 p.m. the rats were cleaned, fed, weighed, etc. In order to secure uniformity of exposure the cages were shifted on a regular schedule, since wax concentrations were somewhat higher at the inflow end of the boxes. Preliminary runs showed that once properly adjusted, wax concentrations in the air remained very uniform from day to day, but to insure absolute safety readings of temperature, air-flow, etc. were made every night between 10:00 and 12:00 p.m. as well as on starting and stopping. At different times during the course of the experiment tests for free chloride were made but were uniformly negative and showed that under the temperatures used no decomposition occurred.

Table 1

NEV 130815

Conditions Maintained in Inhalation Experiment from July 1 to Nov. 18

Material	Temperature	Average concentration of air in box		Total length of exposure	Average daily exposure
	<u>°C</u>	<u>Mg./cu.M.</u>	<u>Range</u>	<u>hours</u>	<u>hours</u>
Chlorinated diphenyl	165-175	0.37	High 1.19 Low 0.23	1896	16

Results of Inhalation Experiments

No abnormalities were seen at any time in the living rats. Blood and urine examinations were made; there were no losses in weight, and one would have considered that the animals were perfectly sound. However, rats sacrificed after 6 weeks exposure showed slight liver damage, which advanced during the next two months. The changes consisted in slight to moderate swelling of the liver cells, increased granularity, and many mitotic figures. Hyaline inclusions were present and were invariably plentiful in animals receiving chlorinated diphenyl by inhalation or by mouth.

One is forced to conclude from the experiments that an average concentration of 0.57 mg. per cubic meter inhaled 16 hours daily produces definite slight changes in the liver and in this organ alone. These changes are resisted efficiently by the animals and cause no depreciation of health. The situation is not unlike that seen in factories where acute yellow atrophy of the liver has occurred. In the case of such instances the patients have been singled out in some way or other from large groups of fellow workmen who have been perfectly healthy.

In considering the entire matter it seemed to us that the chlorinated hydro-carbons, ~~WATER~~ if inhaled in sufficient concentration, might cause a slight degree of damage to the liver. This damage is resisted efficiently and causes no depreciation of health, but if the individual in question happens to suffer some ordinary disease of the liver the condition is superimposed upon a substratum of injury. In accordance with this hypothesis we determined that a dose of 0.75 cc. of carbon tetrachloride plus 0.75 cc. of ethyl alcohol per kilogram of rat was entirely non-toxic to normal animals. When, however, this dose was given to animals which had inhaled chlorinated diphenyl as has been described in this experiment, the result was acute yellow atrophy

NEV 130816

of the liver. It would seem therefore that inhalation of a concentration of chlorinated diphenyl in the manner I have described is capable of producing a condition which may be dangerous to the individual inhaling it, though of itself no visible harm will be done.

Animals removed from exposure to chlorinated diphenyl after two months inhalation and given two months for recovery still showed liver changes when killed and examined, so that it is evident the alterations produced by this hydro-carbon are persistent and individuals who have been caused trouble by it should be removed completely and for a long time.

The Inhalation of a somewhat higher concentration of
chlorinated diphenyl over an 8 hour day

The circumstances attending this experiment are given in Table 2.

Table 2

Material	Temperature	Average concentration of air in box		Total length of exposure	Average daily exposure
	<u>C</u>	<u>Mg./cu.m.</u>	<u>Range</u>	<u>Hours</u>	<u>Hours</u>
Chlorinated diphenyl	153-174	0.93	High 3.23 Low 0.03	920	8

Again 80 rats were used in this experiment. None at any time showed the slightest evidence of illness. Microscopic examinations, however, showed a degree of change very comparable to that found in the 16 hour experiments which have been described and the carbon tetrachloride test was uniformly fatal.

General Summary.

From these experiments we are forced to conclude that concentrations of chlorinated diphenyl in workrooms should not be allowed to rise above 0.5 mg. per cubic meter of air. This concentration is readily obtained by careful

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hooding. It is in our opinion safe for workmen who would not be exposed with the absolute steadiness characteristic of our experiments on rats and such a concentration limit would probably do away with the troublesome skin lesions which have been the particular cause for worry in regard to these compounds in the past.

In addition to the inhalation experiments many feeding experiments were done with ^{this} chlorinated diphenyl. The general changes in the liver were the same as those already described. No diarrhea or gastro-intestinal aspects seemed to be present.

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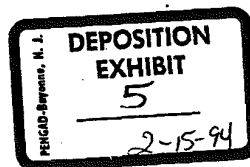
Toxicity

REPORT TO THE MONSANTO CHEMICAL COMPANY

by

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September 15, 1938



NEV 118912

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NEV 118913

I. EXPERIMENTS TO DETERMINE THE POSSIBLE TOXICITY OF THE FOLLOWING SUBSTANCES

1. CHLORCOSANE

On July 14, 1937, we were informed by F. D. Smith that each 6 ounce bottle of a soft drink might contain 60 to 70 mgm. of chlorcosane. There was no pharmacological reason to believe the compound would be irritating or toxic in any way, and therefore it was decided to dose rats with it very heavily, the argument being that if they survived heavy dosage without any evidence of damage a verdict of no toxicity might be given and much time saved. If, on the other hand, the large doses proved poisonous it would be necessary to reduce dosage until a non-toxic level was reached. The first of these alternatives proved correct.

Experiments. -- August 10, 1937. 20 adult white rats ranging in weight from 162 to 210 gm. were each fed 70 mgm. of chlorcosane in 1 cc. of olive oil daily by stomach tube. These animals showed no evidence of damage in any way attributable to chlorcosane. They were examined microscopically from the 35th to the 92nd day. The only constant change observed was a possible slight increase in the granularity of the cells of the liver cords, but the pathologist in charge felt this to be within normal limits. This finding is doubly reassuring since many of the animals developed a chronic suppurative lung condition very prevalent in Boston stock rats at the time. But even with this added handicap there was no evidence of damage and we thus conclude that chlorcosane even in enormous dosage is an inert material in the body and in all probability passes through the intestine unchanged.

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2. DIPHENYL PHTHALATE

On August 23, 1937, we were informed by Dr. R. E. Kelly that diphenyl phthalate might be absorbed through the skin or might be inhaled and swallowed during spraying operations. After consultation it was decided to feed heavily, and if there were indications of toxicity to project other experiments.

Experiments. -- September 20, 1937. 20 adult white rats ranging in weight from 242 to 301 gm. were each fed 0.5 mgm. of diphenyl phthalate suspended in 1 cc. of water by stomach tube daily. The animals were from new stock and with one exception remained clear of the lung condition which existed in the chlorocresane group. During the period of experiment, which lasted 92 days, practically all the animals gained weight. Examination of 8 animals killed at intervals during this period of test resulted as follows:

The organs were normal grossly, and on microscopic examination the liver alone was of possible interest. The changes in the liver varied from those in which the cells of the liver cords were almost normal, having only a slight swelling and granularity of their cytoplasm, to those in which these changes were pronounced. In the latter there was a moderate degree of vacuolization and a rare hyaline body. These alterations it must be understood were the result of very certain dosage, since the compound was given by stomach tube and the animals had no possible way of avoiding it. Neither the pathologist nor myself was able to consider them of enough moment to cause us to make further experiments.

I am confident that with the ordinary precautions accompanying spray lacquering no possible harm could be done, even if the concentration of diphenyl phthalate in the lacquer was far above the 5 per cent figure given us by R. E. Kelly.

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3. CHLORINATED DIPHENYL -- COMPOUND #1268

This material was furnished by the Monsanto Chemical Company. It was said to have a chlorine content of 68 per cent, the highest chlorine figure of any compound tested by us during the past three years. It had been our impression that toxicity and chlorine content followed one another closely, but this has not turned out to be the case for #1268.~~xxxxxxxxxxxxxx~~
~~xxxxxxxxxxxxxx~~ Further discussion of this point will follow the description of the experimental observations and their results.

Experiments. -- August 2, 1937. The compound #1268 was administered by inhalation, the technique being that described in the paper entitled The Problem of Possible Systemic Effects from Certain Chlorinated Hydrocarbons, Journal of Industrial Hygiene, 1937, 19, 283. For the first observations 80 adult white rats were used. They were exposed as follows:

Group 1. -- 80 animals. Temperature to which the compound was heated to introduce fume into air line 140-190° C. Average concentration in air breathed by rats 0.53 mgm. per cubic meter. Average daily exposure 16 hours. The experiment was continued for 119 days, animals being sacrificed at intervals for pathological examination and for the carbon tetrachloride and alcohol test (see paper previously cited).

The conditions described above caused swelling and increase of granularity of the liver cells. Hyaline inclusions were rare. These changes were quite uniform in all animals examined after the 51st day, but were not certainly progressive. The rats were very healthy throughout the period and there was an almost uniform gain in weight. The carbon tetrachloride-alcohol test was positive after 52 days, which indicates that though liver damage was apparently slight some degree of harm had been done the organ. There was no evidence of

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damage in any part of the body except the liver.

At the end of 93 days 10 rats, apparently in excellent condition, were removed from the experiment and set aside for observations upon liver recovery. These recovery animals showed no clinical changes of any sort. When sacrificed 72 and 141 days after removal from exposure it was observed that the swelling of the liver cells had disappeared, but the granular and hyaline material remained in the liver cells and had apparently become permanent. There was absolutely no progression of damage after removal from exposure.

These changes may be compared with those produced in rats by inhalation of chlorinated diphenyl #4465, administered in a similar manner in concentrations of 0.57 to 0.93 mgm. per cubic meter over similar periods of time. In the case of this compound the conspicuous difference was the far greater incidence of hyalinization of the liver cells, which in our opinion was the characteristic lesion caused by #4465 and mixtures containing it. One can therefore conclude that #1268 in low concentrations is definitely less toxic than #4465.

Group 2. — After 119 days, the low concentration of #1268 having proved but slightly poisonous, it was decided to increase the concentration in the air breathed by the 54 rats then remaining. Temperature and other conditions were maintained as during the first 119 days, but by the use of 4 vaporizers instead of 1 the average concentration of #1268 in the air was brought to 6.23 mgm. per cubic meter of air. Exposure to this very high concentration was continued for 87 days.

Under these extreme conditions the animals again remained in perfect health. The carbon tetrachloride and alcohol test was positive but there was absolutely no other indication of liver damage and no evidence of disturbance to other organs.

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When examined at autopsy at the end of 87 days -- the animals had experienced a total exposure of 206 days -- there were no gross evidences of abnormality in any part. On microscopic examination no organ showed changes except the liver. The number of cells containing hyaline and the amount of hyaline in the cells involved increased during this period of exposure to the higher concentration of #1268. The hyaline was most abundant in the portal zone of the lobule with very little in the central area. After 42 days of exposure 10 rats were set aside and were sacrificed 73 days later. They were in excellent condition when removed and did not change. Grossly, at autopsy, they showed nothing abnormal, and on microscopic examination the liver cells had lost their swelling but retained increased granularity and hyaline inclusions.

The experiments on inhalations of high concentrations of #1268 reenforced the conclusion that this compound is of low toxicity as compared with #4465 or with chlorinated naphthalenes above trichloronaphthalene. The question as to why #1268, the most highly chlorinated compound tested, proved but slightly harmful cannot be answered with any definiteness. It has been suggested that the toxicity of all these chlorinated compounds, even though of varied composition, may depend on the ability of the animal to decompose them after lodgment in the tissues, and that this decomposition might be shown by an increase in the chlorine in the urine in suitably conducted feeding experiments. In experiments upon this point we have shown a definite increase in the urinary chlorides when dogs and rats were fed a mixture of penta and hexachloronaphthalenes (#1006), a compound highly injurious to the liver and containing 62.6 per cent of chlorine, but similar observations have not been made with any of the chlorinated diphenyls or allied compounds. It may, however, be that when about 65 per cent chlorination is reached the substances formed are quite stable in the body and so cause a minimum of damage.

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In conclusion, #1268, if handled with ordinary precautions as to ventilation should be entirely harmless to workmen. While it cannot be given an absolutely clean bill as to health, it is preferable to #4465 and #5460.

4. MIXTURE OF CHLORINATED DIPHENYL AND CHLORINATED DIPHENYL

BENZENE -- COMPOUND #5460

This substance was furnished by the Monsanto Chemical Company and was said to have a chlorine content of 60 per cent, being in this respect below #4465 and far below #1268.

Experiments. -- The inhalation technique was used as in the case of #1268 and #4465.

August 2, 1937. 80 adult white rats were the subjects. Temperature to which the compound was heated to introduce fume into the air line 140-150° C. Average concentration in air breathed by rats 0.085 mgm. per cubic meter. Average daily exposure 16 hours. The experiment lasted 119 days. A certain number of animals were sacrificed for pathological examination, others were used for the carbon tetrachloride and alcohol test, and still others set aside for observations as to recovery from possible damage.

In spite of the fact that the concentration in the air breathed by the rats averaged about 1/6 that obtained at comparable temperature as from #1268, a number of these animals became sick and lost weight towards the end of the second month of exposure. When killed, such individuals showed gross mottling of the liver but no changes in other organs. On microscopic examination swelling of the liver cells, increased granularity and hyaline inclusions were noted in animals killed as early as the 16th day of exposure. These changes obviously occurred rapidly. Hyaline deposits were never as numerous as with #1268. Animals

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removed for recovery after 51 and 119 days of exposure did not gain markedly in weight, and one died for no obvious cause. When examined at autopsy the swelling of the liver cells had subsided, but abnormal granularity and hyalinization remained.

Needless to say the carbon tetrachloride and alcohol test was positive whenever used.

In view of the fact that #5460 in such low concentration proved so definitely toxic, no higher concentrations were tested. It seems imperative that whenever this compound is used in industry, great care be taken to keep concentrations in the air at an extremely low level. No liberties can be taken with it, as with #1268.

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II. SOME GENERAL CONSIDERATIONS

In addition to these tests of compounds, certain experiments were done which are of interest to those manufacturing or using chlorinated hydrocarbons. Details as to these experiments are of course available but are not included in this report since they are not of direct industrial interest.

1. EVIDENCE FOR THE DESTRUCTION OF A MIXTURE OF PENTA AND HEXACHLORONAPHTHALENES (#1006) IN THE BODY

It has always been a question as to whether the chlorinated hydrocarbons which have been examined by ourselves and by others do harm per se or whether toxicity depends on their breakdown in the body with the liberation of something harmful to the liver. A partial answer has been obtained by feeding #1006 to rats and dogs which were on a low chloride diet with uniform excretion of chlorides in the urine. When these animals received the chlorinated hydrocarbon (#1006) the urinary chlorides rose. This indicates that the body certainly has power to detach chlorine from this compound, and it is probable that the same condition is true for allied toxic compounds. It would be most interesting to see whether ingestion of #1268 results in similar findings or whether in the case of this relatively non-toxic compound there is practically no splitting off of chlorine. Neither time nor our financial resources permitted such tests.

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2. THE EFFECT OF INCREASING THE SODIUM CHLORIDE IN THE DIET
UPON ANIMALS RECEIVING TOXIC DOSES OF #1006

On the ground that chlorides might be fundamentally associated with toxicity, a group of 15 rats was fed a low toxic dose of #1006 and compared with a similar group on the same dosage of #1006 plus a marked increase in chloride intake secured by giving 5 cc. per kilogram of body weight of 4 per cent NaCl solution daily.

No differences were found between the two groups, and it may be concluded that chlorine increase secured through the diet does not enhance toxicity. This experiment was done in order to find out whether increase in chloride intake during hot weather might be harmful.

A particular phase of the problem, the possible enhancement of typical skin lesions by increasing chloride intake, cannot be decided by experiments on fur-bearing animals with no sebaceous glands. All that can be said at the moment is that increased chloride intake does not increase systemic toxicity.

3. THE EFFECT OF HIGH AND LOW CALCIUM DIETS ON ANIMALS
RECEIVING HIGH CONCENTRATIONS OF #1006

It is well known that a diet rich in calcium is markedly effective in preventing the acute yellow atrophy of the liver produced by carbon tetrachloride (The Prevention and Treatment of Carbon Tetrachloride Intoxication. By P. D. Larson, M.D., A. S. Minot, Ph.D., and D. H. Robbins, M.S., Journal of the American Medical Association, 1928, volume 90, page 345).

To discover whether calcium in the diet would protect against liver damage

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from a toxic chlorinated hydrocarbon, 30 adult white rats were placed upon a diet of lean horsemeat, starch and lard, a combination adequate for maintenance but very low in calcium. Another group was given a diet consisting of dog chow, milk, lettuce and eggs, with added calcium lactate -- a ration very high in calcium.

Both groups were exposed simultaneously to inhalation of high concentrations of #1006, an average of 11.21 mgm. per cubic meter for 16 hours a day.

After 16 days, 12 high calcium diet rats were alive and 7 of the low calcium group. At intervals animals were killed for examination. In both groups the liver was abnormal grossly and microscopically, and in both groups animals died from liver damage. It was impossible to consider that the high calcium diet was in the least degree protective. It may, therefore, be concluded that adding calcium to the diet of workers either in the form of extra milk or of calcium lactate will not prevent liver damage.

4. THE EFFECT OF INJECTIONS OF XANTHINE ON ANIMALS INHALING HIGH CONCENTRATIONS OF #1006

In 1937, R. C. Neale published a brief paper (The Protective Action of Certain Purines against Liver Necrosis Produced by Carbon Tetrachloride and Chloroform. Science, 1937, volume 86, page 83). He claimed that rats injected with sodium xanthine became markedly resistant to carbon tetrachloride. This suggested that xanthine might have similar protective power over liver damage from the chlorinated hydrocarbons on examination in this laboratory.

Accordingly 38 adult white rats were caused to inhale #1006 in concentrations averaging 15 mgm. per cubic meter for 16 hours daily. One group of 20 rats was given 20 mgm. of xanthine subcutaneously every other day and 40 mgm.

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of xanthine by stomach tube on the alternate days. The second group of 18 rats had the same exposure to #1006 without xanthine treatment. No differences were noted and one cannot expect any efficacy from xanthine either in the prevention or treatment of liver disease due to this chlorinated hydrocarbon, and in all probability the same negative result would be encountered in connection with allied toxic compounds.

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THE JOURNAL OF INDUSTRIAL HYGIENE AND TOXICOLOGY

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NUMBER 5

FURTHER OBSERVATIONS ON THE POSSIBLE SYSTEMIC TOX- ICITY OF CERTAIN OF THE CHLORINATED HYDROCARBONS WITH SUGGESTIONS FOR PERMISSIBLE CONCENTRA- TIONS IN THE AIR OF WORKROOMS*

CECIL K. DRINKER

Department of Physiology, Harvard School of Public Health, Boston, Mass.

SINCE 1937, several papers have appeared dealing with the systemic effects of certain of the chlorinated hydrocarbons. These are listed in the bibliography in the order of their appearance (1, 2, 3, 4, 5). The first of these papers included recommendations as to safe concentrations in the air of workrooms, that is, concentrations that would cause no ill effects to individuals who breathed them throughout a working day. It also mentioned certain other observations, such as the effects of diet on toxicity, which were being made at the time but which had not progressed far enough to merit description. It is now possible to report upon these matters and to list a fairly large number of chlorinated hydrocarbons together with permissible concentrations in air.

* Received for publication February 10, 1939.

THE EFFECT OF HIGH AND LOW CALCIUM INTAKE ON ANIMALS IN-
HALING HIGH CONCENTRATIONS OF A
MIXTURE OF PENTA AND HEXA-
CHLORNAPHTHALENES (CHLORINE
62.6%).

It is well known that a diet rich in calcium is markedly effective in preventing the acute yellow atrophy of the liver produced by carbon tetrachloride (Lamson et al. (6)). To discover whether calcium in the diet would protect against liver damage from a toxic chlorinated hydrocarbon, 30 adult white rats were placed upon a diet of lean horsemeat, starch and lard, a combination adequate for maintenance but very low in calcium. Another group was given a diet consisting of dog chow, milk, lettuce and eggs, with added calcium lactate,—a ration very high in calcium. Both groups were exposed simultaneously

DEPOSITION
EXHIBIT

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to inhalation of high concentrations of a mixture of penta and hexachloronaphthalenes (chlorine 62.6%), an average of 11.21 mg. per cu.m. for 16 hours a day.

After 16 days, 12 high calcium diet rats were alive and 7 of the low calcium group. At intervals animals were killed for examination. In both groups the liver was abnormal grossly and microscopically, and in both groups animals died from liver damage. It was impossible to consider that the high calcium diet was in the least degree protective. It may, therefore, be concluded that adding calcium to the diet of workers either in the form of extra milk or of calcium lactate will not prevent liver damage.

THE EFFECT OF INJECTIONS OF XANTHINE ON ANIMALS INHALING HIGH CONCENTRATIONS OF A MIXTURE OF PENTA AND HEXACHLORNAPHTHALENES (CHLORINE 62.6%).

In 1937, R. C. Neale (7) published a brief paper in which he claimed that rats injected with sodium xanthine became markedly resistant to carbon tetrachloride. This suggested that xanthine might have similar protective power over liver damage from the chlorinated hydrocarbons on examination in this laboratory. Accordingly 38 adult white rats were caused to inhale a mixture of penta and hexachloronaphthalenes (chlorine 62.6%) in concentrations averaging 15 mg. per cu.m. for 16 hours daily. One group of 20 rats was given 20 mg. of xanthine subcutaneously every other day and 40 mg. of xanthine by stomach tube on the alternate days. The second group of 18 rats had the same exposure to the compound without xanthine

treatment. No differences were noted and one cannot expect any efficacy from xanthine either in the prevention or treatment of liver disease due to this chlorinated hydrocarbon, and in all probability the same negative result would be encountered in connection with allied toxic compounds.

EVIDENCE FOR THE DESTRUCTION OF MIXTURE OF PENTA AND HEXACHLORNAPHTHALENES (CHLORINE 62.6%) IN THE BODY.

It has always been a question as to whether the chlorinated hydrocarbons which have been examined by ourselves and by others do harm *per se* or whether toxicity depends on their breakdown in the body with the liberation of something harmful to the liver. A partial answer has been obtained by feeding the above compound to rats and dogs which were on a low chloride diet with uniform excretion of chlorides in the urine. When these animals received the chlorinated hydrocarbon the urinary chlorides rose. This indicates that the body certainly has power to detach chlorine from this compound, and it is probable that the same condition is true for allied toxic compounds.

THE EFFECT OF INCREASING THE SODIUM CHLORIDE IN THE DIET UPON ANIMALS RECEIVING TOXIC DOSES OF A MIXTURE OF PENTA AND HEXACHLORNAPHTHALENES (CHLORINE 62.6%).

On the ground that chlorides might be fundamentally associated with toxicity, a group of 15 rats was fed a low toxic dose of this compound and compared with a similar group on the same dosage plus a marked increase in

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ences were found between the two systemic toxicity.

TABLE 1

A LIST OF 14 CHLORINATED HYDROCARBONS, WITH CHLORINE CONTENTS AND PERMISSIBLE
LIMITS (IN MG./CU.M.) FOR THE AIR IN WORKROOMS*

COMPOUND	CHLO- RINE CONTENT	PERMI- SSIBLE LIMIT
	%	mg./ cu.m.
1. Trichloronaphthalene plus a trace of tetrachloronaphthalene. Tested upon rats by inhalation and by feeding.....	49.9	10.0
2. Tetra and pentachloronaphthalenes. Tested upon rats by inhalation and by feeding.....	56.4	1.0
3. Penta and hexachloronaphthalenes. Tested upon rats by inhalation and by feeding, and upon dogs by feeding alone.....	62.6	0.5
4. Tetra and pentachloronaphthalenes plus refined chlorinated diphenyl. Tested upon rats by feeding.....	43.5	0.5
5. 90% penta and hexachloronaphthalenes plus 10% chlorinated diphenyl benzene. Tested upon rats by inhalation and by feeding.....	63.0	0.5
6. Chlorinated diphenyl plus chlorinated diphenyl benzene. Tested upon rats by inhalation and by feeding.....	65.0	0.5
7. Chlorinated diphenyl oxide. Tested upon rats by inhalation.....	54.0	0.5
8. Chlorinated diphenyl oxide. Tested upon rats by inhalation.....	57.0	0.5
9. Chlorinated diphenyl. Tested upon rats by inhalation.....	50-55	0.5
10. Hexachlor diphenyl oxide plus 5% trichloronaphthalene. Tested upon rats by inhalation.....	50-55	0.5
11. Hexachloronaphthalene and crude chlorinated diphenyl. Tested upon rats by inhalation.....	Un- known	0.5
12. Special chlorinated naphthalene. Tested upon rats by inhalation.....	50-56	0.5
13. Chlorinated diphenyl. Tested upon rats by inhalation.....	68	10.0
14. Chlorinated diphenyl benzene. Tested upon rats by inhalation.....	60	0.5

* The analytical method and apparatus used routinely for field determinations is that described by Tebbens (THIS J., 19, 204 (1937)) and by Drinker *et al.* (ibid., p. 283).

groups, and it may be concluded that
chlorine increase secured through the
diet does not enhance toxicity. This
experiment was done in order to find
out whether increase in chloride intake
during hot weather might be harmful.

A particular phase of the problem,
the possible enhancement of typical
skin lesions by increasing chloride in-
take, cannot be decided by experi-
ments on fur-bearing animals with no

THE SYSTEMIC EFFECTS OF 14 CHLO- RINATED HYDROCARBONS WITH SUG- GESTED LIMITS FOR CONCENTRA- TIONS IN THE AIR OF WORKROOMS.

The technic for inhalation and
feeding experiments upon rats has
been fully described (Drinker *et al.*
(1)). It has been uniform for all the
compounds listed in table 1, and for
each group of experiments 24 to 48

animals have been sacrificed and examined grossly and microscopically so as to gain very complete post-mortem reports.

The first 6 substances in table 1 have been dealt with in previous published reports (cf. 1 and 3 in bibliography). In connection with them it was recommended that in the case of trichloronaphthalene and compounds of lower chlorination a limit of 10 mg. per cu.m. be permitted, the idea being that the essential feature of the action of these compounds, namely damage to the liver, did not become prominent until a chlorine content of 50% was reached. So far as trichloronaphthalene is concerned the limit of 10 mg. should be allowed to stand, since nothing has occurred which indicates the necessity for changing it. The second compound, a mixture of tetra and pentachloronaphthalenes is in common industrial use. A tolerance of 0.5 mg. per cu.m. was formerly proposed for it, but on the basis of laboratory and field experience it is apparently entirely safe to increase this to 1.0 mg. per cu.m.

The sixth compound has been listed previously as chlorinated diphenyl. It contained 65% of chlorine and proved very destructive to the liver. Later experiments with compound 13, which contained 68% of chlorine and which was also labelled chlorinated diphenyl, were a surprise to us since this second compound was almost non-toxic. On inquiry it was found that substance 6 was in reality a mixture of chlorinated diphenyl and chlorinated diphenyl benzene and that number 13 was actual chlorinated diphenyl. We have no information as to whether this last compound lacks

toxicity because it is not broken down in the body, but that would seem the probable explanation. Compound number 12 was prepared especially in order to have a substance for test which should fall in chlorination between compounds 1 and 2. It proved somewhat toxic and, in addition to changes in the liver, caused eye irritation and in one instance cataracts. The substance is not in commercial use and evidently at the temperatures used for volatilization gave off some sort of irritant, possibly hydrochloric acid.

With the exception of this isolated effect upon the eyes, we have seen no lesions from any of the 14 compounds listed except in the liver. The character of these lesions has been thoroughly described by Bennett *et al.* (3). These additional data and the tolerances recommended deal specifically with systemic effects since one can get no information as to chloracne in the skin from observations upon fur-bearing animals. But it may be pointed out that where industrial workers have been careful as to cleanliness and where air concentrations have been kept within the limits suggested, skin lesions apparently cease to occur.

SUMMARY

Certain experiments carried out upon large groups of white rats indicate the following:

1. A high calcium diet has no protective effect against the characteristic liver lesions caused by these compounds.
2. Administration of xanthine does not prevent liver injury.

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3. When a mixture of penta and hexachloronaphthalene is given to dogs by mouth, the urinary chlorides rise, indicating that in some way the body cells detach chlorine from this compound.

4. A high chlorine intake does not enhance toxicity.

5. A tabulation can now be presented covering 14 chlorinated hydrocarbons with permissible safe concentrations in the air of workrooms.

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ients. Thyroid function may be depressed in a higher percentage of cases if urethane therapy is maintained for a longer period. This possibility is currently being investigated.

SUMMARY

Twenty patients were treated with rectal suppositories of urethane. The therapeutic effectiveness of rectally ad-

ministered urethane is equivalent to that of orally administered urethane. Urethane administered rectally is as effective as Fowler's solution in spacing irradiation therapy. Undesirable side-effects were minimal. It is concluded that rectal administration of urethane is preferable to oral or intravenous administration.

2065 Adelbert Rd. (6) (Dr. Weisberger).

CHLORACNE FROM AN UNUSUAL EXPOSURE TO AROCHLOR

J. Wister Meigs, M.D., Jack Jonathan Albom, M.D.

and

Bernard L. Kartin, M.D., New Haven, Conn.

The first outbreak of acne-like lesions due to high boiling chlorinated compounds in industry in this country was noted by Schwartz¹ in 1936. Subsequently, during World War II, various reports of acne-like lesions in workers exposed to certain chlorinated naphthalenes and diphenyls followed. Collier² reported 12 cases of chloracne of the face in workers exposed to the fumes or dust of chlorinated naphthalene and one fatal case due to acute yellow atrophy of the liver. Fifty-five cases of acne-form dermatitis were reported by Kelley³ in 200 persons exposed to chlorinated naphthalene (Halowax). Good and Pensky⁴ described 52 cases in electricians, who handled the cold-finished product for the most part. In none of their cases was systemic involvement found.

Swartz's report¹ concerned the involvement of electricians, who installed and stripped wires in ships during the war. The chlorinated naphthalene, which was impregnated into the asbestos and wrapped around the wire as insulation, flaked off in the stripping process. In the cases described, two months elapsed before the appearance of the chloracne of the face. There was no systemic involvement. Peck,⁵ Cranch,⁶ and Greenburg⁷ discuss the chlorinated naphthalenes and chlorinated diphenyls, the appearance of the dermatological lesions, their value in industry, and precautions in handling these chemicals.

The value of the chlorinated naphthalenes and diphenyls in industry is due primarily to their resistance to water and alkali, high insulating value (high dielectric constant), thermoplasticity, chemical stability, and flame resistance, as described by various writers.⁸

The characteristic lesions of chloracne are pinhead to pea-sized pale straw-colored cysts formed by the plugging of the orifices of the sebaceous glands, resulting in retention of the secretion and in the keratinization of the lining membrane. Comedones are present but are not a striking feature. In nearly every worker exposed sufficiently to these chlorinated compounds for a few months these lesions will develop. The exposure may be either to fumes from the hot material or to the solid material on continued contact. Repeated or continuous contact is essential. Lesions have not been reported after short or infrequent exposures. Vesiculoerythematous eruptions, as seen in acute eczematous contact-type dermatitis and simple erythematous eruptions with pruritus, have also been described.⁹

This paper reports the development of lesions of chloracne in seven workers employed in a chemical plant concerned with organic chemical production.

HISTORY OF EXPOSURE

A chemical company had for some months been using molten salt at 350 F as a medium for supplying accurately controlled quantities of heat to a large jacketed reaction chamber. Because of the dangers of solidification of the salt in the return line, as well as the corrosion problem, it was decided to use a chlorinated diphenyl (Arochlor) as the heat exchange material. The same apparatus was used. This included an oil-fired furnace containing the heating coils, a steel pipe supply line to the reaction chamber, a return line to a large sump pump, and an outflow line from the sump to the furnace (see figure). The reservoir for the sump pump had a capacity of about 400 gal. (about 1,680 liters).

The chemical product, designated as organic acid A, was manufactured with the use of molten salt for heat exchange from January to December, 1949. At that time, chlorinated diphenyl was substituted as a heat exchange material. It was soon apparent that under certain conditions there was slight leakage of vapors from a number of places, particularly around the cover of the sump, and also from all gasketed connections in the system. Because of the known toxicity of these substances, the assistance

From the departments of public health and internal medicine, Yale University School of Medicine.

Associate Professor of Occupational Medicine, Department of Public Health (Dr. Meigs), Assistant Clinical Professor of Dermatology, Department of Internal Medicine (Dr. Albom), and Assistant Clinical Professor of Medicine, Department of Internal Medicine (Dr. Kartin), Yale University School of Medicine.

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2. Collier, E.: Poisoning by Chlorinated Naphthalene, *Lancet* 1: 72 (Jan. 16) 1943.

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6. Peck, S. M.: Dermatitis from Cutting Oils, Solvents, and Dielectrics, Including Chloracne, *J. A. M. A.* 125: 190 (May 20) 1944.

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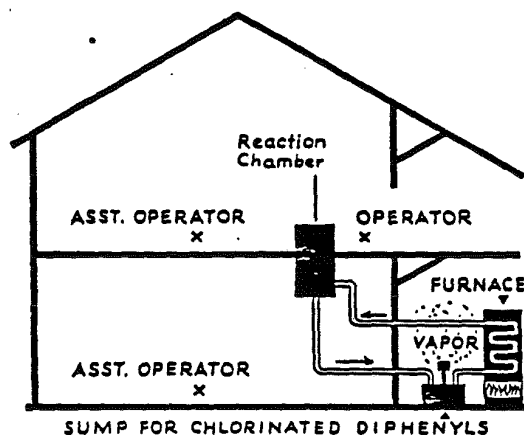
9. (a) Mayers, M. R., and Silverberg, M. G.: Skin Conditions Resulting from Exposure to Certain Chlorinated Hydrocarbons, *J. Indust. Hyg. & Toxicol.* 20: 244 (March) 1938. (b) Schwartz.¹ (c) Greenburg.⁷

DEPOSITION
EXHIBIT

AP-Bayonne, N. J.

7

of the Bureau of Industrial Hygiene, Connecticut State Department of Health, was sought and received. A field study under conditions of obvious vapor leakage was said to have shown negligible air concentrations of the chlorinated diphenyls in the actual breathing zones of the workers. In this study, which was four months prior to the dermatological findings reported here, the air concentration of chlorinated diphenyls was reported by the bureau to be 0.1 mg. per cubic meter of air. The recommended maximum allowable concentration is 1.0 mg. per cubic meter. The figure shows that most of the leakage was at points outside the building but under a roof. No one worked regularly at the points of leakage. Nevertheless, repeated attempts were made to control vapor leakage, without complete success. This operation continued for 19 months without incident or recognition of skin or other manifestations of exposure to chlorinated diphenyls. Each employee had a complete physical examination by an internist prior to working in this environment.



Schematic drawing of the heat exchange system in which the chlorinated diphenyl was used and the points at which workers were exposed to fumes.

An operator making organic acid A ("operator," figure) was sent to one of us because of acute contact dermatitis of the face. In addition to the contact-type of dermatitis, there were noted pinhead-sized straw-colored cysts and comedones on both cheeks and the forehead. A diagnosis of chloracne was made, and the source of exposure was determined. Examination of other workers in this working environment uncovered six additional cases. In all the face was involved especially the cheeks circumorbitally, the forehead, ears, and in one case the mastoid region. All employees were examined carefully by an internist. The seven employees in whom chloracne had developed had liver function tests performed. Tests included direct and total bilirubin determinations and 24 and 48 hour cephalin flocculation, thymol turbidity, and alkaline phosphatase determinations. Six of the subjects had completely normal test results. One employee had borderline cephalin flocculation and thymol turbidity. Thirteen months later repeated liver function tests showed an unchanged cephalin flocculation and improved thymol turbidity. Results of complete blood cell

counts and urinalysis were normal in all instances. All had normal blood pressures with no other clinical evidence of any chlorinated diphenyl toxicity.

After the recognition of these cases of chloracne, all but one of the gasketed joints in the heat exchange system, including the cover of the sump, were welded together. A hand hole 6 in. (15 cm.) in diameter was left with a gasketed cover so that the system could be drained or filled when necessary. After that time no vapors were visible, and the odor of chlorinated diphenyls was barely detectable in the immediate vicinity of the sump pump. Continued careful observation of workers has revealed no new cases of chloracne.

COMMENT

The unusual feature of this outbreak of dermatitis was the long period of exposure before any cases were recognized. The sudden recognition of seven cases after exposure up to 19 months was due to the especially careful examination of the skin of all exposed employees after discovery of the first case. Of 14 men exposed or potentially exposed to the vapors of chlorinated diphenyls, 7 presented clinical evidence of chloracne. There was not a very good correlation between the apparent degree of exposure and the development of signs of disease. For example, a foreman, an assistant foreman, and a plant superintendent whose duties would appear to have exposed them only incidentally to the toxic agent had mild to moderate signs. The mean length of exposure of those in whom signs developed was 14.3 months and of those who did not show signs was 11.4 months, but there was considerable overlap, with chloracne developing in one worker after only 5 months in contrast to another who showed no signs even after 19 months' exposure to vapors. Since the manifestations were exclusively on exposed areas of the skin, it appears that the vapors were deposited directly on exposed skin and did not go through the clothing. The nature of each factor determining the appearance or nonappearance of lesions is not clear. Skin pigmentation may be a factor. Three of the workers were Negroes, and none of them had chloracne. No correlation with perspiration could be made, but all cases were discovered toward the end of the summer.

Prevention consisted of controlling the leakage of vapors. The fact that tests of the air, even in the presence of vapors, showed only negligible amounts of chlorinated hydrocarbons indicates that this type of intermittent but fairly long continued "mild" exposure is not innocuous. The low concentration of the chlorinated diphenyl in the air might account for the fact that lesions developed in only 50% of those involved.

SUMMARY

Seven cases of mild to moderate chloracne of the face and head occurred among 14 chemical operators exposed from 5 to 19 months intermittently to small concentrations of the vapors of a chlorinated diphenyl (Arochlor). Leakage of these vapors from a heat exchange system occurred chiefly outdoors, but chloracne was observed among men working inside the adjacent building. In all cases the condition cleared up after treatment. Control of vapors by welding all joints in the heat exchange system prevented recurrences.

310 Cedar St. (Dr. Meigs).

MONSANTO CHEMICAL COMPANY

ST. LOUIS 4, MISSOURI

C O P Y

April 28, 1954

C O P Y

Dr. J. Wister Meigs
310 Cedar Street
New Haven, Connecticut

Dear Dr. Meigs:

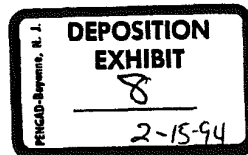
Your article in the current number of the JAMA is certainly confusing to me. We have had men exposed to concentrations of chlorinated diphenyl four or five times what you have recorded for periods of five to ten years and have had no chloracne.

Could you tell me which Aroclor was used? I notice you have referred to the product they manufactured as organic acid A. Are you at liberty to tell me what that product was? If you are not, does it contain any chlorinated compound or is any chlorinated compound used as an intermediate? Is organic acid A the only product this plant manufactures? And if not, are any other chlorinated compounds, such as penta-chlorophenol, 2,4,5-T, 2,4-D, or DDT manufactured?

Very truly yours,

R. Emmet Kelly, M.D.
Medical Director

REK:MFL



NEV 160086

YALE UNIVERSITY SCHOOL OF MEDICINE
DEPARTMENT OF PUBLIC HEALTH

SECTION OF
OCCUPATIONAL HEALTH

310 CEDAR STREET
NEW HAVEN 11, CONNECTICUT

May 7, 1954

R. Emmet Kelly, M.D.
Medical Director
Monsanto Chemical Company
St. Louis 4, Missouri

Dear Doctor Kelly:

Thanks for your letter of April 28th. Our observations of chloracne (mostly quite mild) were confusing to me too. That was one reason for publishing the data, since there is a tendency among all of us to assume that certain conditions can be characterized as "safe" on the basis of environmental studies alone. None of us here (including the State Health Department Survey people) have any good answer to why the air samples were so low. We assume that somehow other conditions may have existed for a few weeks or months prior to the recognition of chloracne.

Organic acid A did not contain chlorine, nor did anything else in the particular building. However, the product may have played a part, although by itself it acts only as an irritant (it is packaged as a powder). The Arochlor was from one of the large suppliers and was a combination of high boiling chlorinated hydrocarbons. Since the repairs on the circulating system there have been no more cases - over a three year period.

My conclusion is that chlorinated hydrocarbons should ultimately be judged as safe or hazardous in relation to observations of workers themselves. Study of the environment would seem at best to be only a guide.

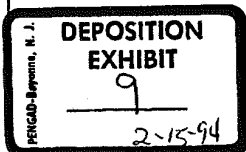
One of our purposes was to stimulate interest in occupational medicine. I hope the article will not cause a rash of claims. It seemed to us that the diagnosis of chloracne was so specific that any competent dermatologist could make it. Therefore, disputed cases should not be frequent. On the other hand, if even mild chloracne is occurring, management representatives would want their doctors to be alert to it, as it would seem to be a preventable condition.

Finally, I am not suggesting that the M.A.C. for Arochlor has been set too high. It will take years of observation and experience to determine this. Our "outbreak" may have been an isolated experience not likely to be duplicated. In that connection, it would be helpful and informative if you could publish your experience on exposures over long periods to these substances. We need many more reports to have a basis for sound judgement.

With best regards to you and Elmer Wheeler.

NEV 160085

Sincerely yours,
Walter M. Klig
J. Walter Klig, M.D.
Associate Professor of



JW:mafa

Scientific Associates

1474 S. VANDEVENTER AVE. • ST. LOUIS 10, MO.

November 10, 1953

CERTIFICATE OF ANALYSIS

C401-10

SUBJECT -

The Acute Oral Toxicity (LD_{50}) of Aroclor 1254 For Rats.

TEST CONDUCTED FOR -

Monsanto Chemical Company, St. Louis, Missouri.

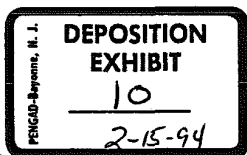
Monsanto Project No.: SA-15

EXPERIMENTAL PROCEDURE -

The LD_{50} was determined by the introduction of measured single doses of undiluted Aroclor 1254 into the stomachs of Sprague Dawley strain albino rats by means of a rubber catheter attached to a hypodermic syringe. Animals weighing 175 to 250 grams were used in the test.

After the approximate Minimum Lethal Dose was found, groups of animals were fed at levels designed to blanket the toxicity range thereby supplying data for calculation of the LD_{50} which was done according to a modification of the method of Bliss (Quart. J. Pharm. and Pharmacol., 11, 1938). The data based on results obtained from a total of 41 rats are found in Table I.

Selected animals from those receiving a lethal dose were autopsied and macroscopic examination made of the viscera.



NEV 017288

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CONSULTANTS TO THE FOOD, CHEMICAL AND PHARMACEUTICAL INDUSTRIES

TO: Monsanto Chemical Company
St. Louis, Missouri

Scientific Associates Certificate of Analysis -- Page 2 (11-10-53)

TABLE I

THE LD₅₀ OF AROCLOR 1254 FOR RATS

<u>Animal No.- Sex</u>	<u>Weight Grams</u>	<u>Dose Ml./Kg.</u>	<u>Fate</u>
1- Male	225	2.50	Survived
2- Male	115		Died
3- Male	240		Survived
4- Female	185		Survived
5- Female	195		Survived
6- Male	220		Died
7- Female	200		Died
8- Female	175		Survived
9- Female	190		Survived
10- Male	230		Survived
11- Male	250	3.00	Died
12- Male	215		Died
13- Female	205		Survived
14- Female	190		Survived
15- Female	180		Survived
16- Male	210		Died
17- Male	230		Survived
18- Male	240		Survived
19- Male	225		Died
20- Female	185		Survived
21- Female	195	3.25	Died
22- Female	205		Died
23- Male	240		Survived
24- Male	230		Survived
25- Male	215		Died
26- Male	245		Died
27- Female	200		Survived
28- Female	180		Died
29- Male	190		Died
30- Female	215		Died
31- Female	205	3.75	Survived
32- Female	190		Died
33- Male	240		Died
34- Male	230		Survived
35- Male	245		Died
36- Female	180		Survived
37- Female	190		Died
38- Female	185		Died
39- Male	225		Died
40- Male	210		Died
41- Male	240		Died

NEV 017289

TO: Monsanto Chemical Company
St. Louis, Missouri
Scientific Associates Certificate of Analysis -- Page 3 (11-10-53)

DISCUSSION -

The LD₅₀ was calculated to be 3.10 milliliters per kilogram with upper and lower limits of 2.98 to 3.24 milliliters per kilogram. Survival time ranged from 1 to 6 days with the majority of deaths occurring in 2 to 3 days. There was moderate diarrhea, lethargy, and poor appetite in all of the animals.

At autopsy the liver varied from a straw color to dark red in different viscera. The intestinal tract was irritated with mucus present in several instances.

SUMMARY -

The LD₅₀ of Aroclor 1254 for rats was found to be 3.10 milliliters per kilogram. Fiducial limits were 2.98 to 3.24 milliliters per kilogram.

SCIENTIFIC ASSOCIATES

Fred M. Younger
BY: FRED M. YOUNGER

NEV 017290

SA-19

Scientific Associates

1474 S. VANDEVENTER AVE. • ST. LOUIS 10, MO.

December 11, 1953

CERTIFICATE OF ANALYSIS

C+25AM

SUBJECT -

The Acute Oral Toxicity (LD_{50}) of Aroclor 1242 For Rats.

TEST CONDUCTED FOR -

Monsanto Chemical Company, St. Louis, Missouri.

Monsanto Project No.: SA-19

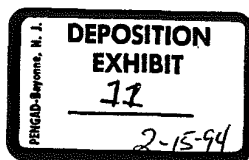
Monsanto Sample No.: Lot No. 166

EXPERIMENTAL PROCEDURE -

The Acute Oral Toxicity (LD_{50}) was found by the introduction of measured single doses of undiluted Aroclor into the stomachs of Sprague Dawley strain albino rats by means of a rubber catheter attached to a hypodermic syringe.

After the toxicity range was found, groups of animals were dosed at levels designed to blanket the toxicity range thereby supplying data for calculation of the LD_{50} , which was done according to a modification of the method of Bliss (Quart. J. Pharm. and Pharmacol., 11, 1938).

Selected animals from those receiving a lethal dose were autopsied and macroscopic examination made of the viscera. The data are given in Table I.



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CONSULTANTS TO THE FOOD, CHEMICAL AND PHARMACEUTICAL INDUSTRIES

T A B L E 1

THE ACUTE ORAL TOXICITY (LD₅₀)
 OF AROCLOR 1242 FOR RATS

Animal No.- Sex	Weight Grams	Dose Ml./Kgs.	Fate
1- Female	185	3.25	Survived
2- Female	170		Survived
3- Female	190		Survived
4- Male	230		Died
5- Male	215		Died
6- Female	200		Survived
7- Male	210		Died
8- Male	235		Survived
9- Male	240		Died
10- Female	195		Survived
11- Female	185	3.75	Survived
12- Male	245		Survived
13- Female	220		Died
14- Male	215		Died
15- Male	235		Survived
16- Female	180		Survived
17- Male	190		Survived
18- Male	225		Died
19- Female	210		Survived
20- Female	230		Survived
21- Female	175	4.25	Died
22- Male	220		Survived
23- Male	240		Died
24- Male	245		Died
25- Female	200		Survived
26- Female	185		Survived
27- Female	190		Survived
28- Male	230		Died
29- Male	220		Survived
30- Male	250		Survived
31- Male	195	4.75	Died
32- Female	210		Died
33- Female	180		Died
34- Female	175		Died
35- Male	220		Died
36- Male	230		Survived
37- Male	200		Survived
38- Male	240		Died
39- Female	185		Died
40- Female	200		Died
41- Female	175		Survived
42- Female	180		Died
43- Female	190		Died
44- Female	175		Died

NEV 017292

TO: Monsanto Chemical Company
St. Louis, Missouri
Scientific Associates Certificate of Analysis -- Page 3 (12-11-53)

DISCUSSION •

The LD_{50} was calculated to be 4.15 milliliters per kilogram with fiducial limits of 4.02-4.36 milliliters per kilogram. Survival time for those animals receiving a lethal dose varied from 12-54 hours.

Lethargy developed in the majority of animals a few hours after dosing. There was also moderate diarrhea.

At autopsy, the liver was dull red with a greenish hue while the spleen was abnormally dark. The kidneys appeared normal by macroscopic examination.

SUMMARY -

The LD_{50} of Aroclor 1242 was found to be 4.15 milliliters per kilogram with lower and upper limits of 4.02-4.36 milliliters per kilogram.

SCIENTIFIC ASSOCIATES

Fred M. Younger

BY: FRED M. YOUNGER

This report is to be used in development of the product and may be given to responsible sales contacts, but it is not to be used by them in advertising copy. The source of this material is not to be divulged until it appears in formal publications. No exceptions to the established rule may be made without the approval of the Medical Department in St. Louis. Customers' inquiries regarding matters of toxicity are to be referred as before to the Medical Department in St. Louis for reply.

— Monsanto Chemical Company

NEV 017293

✓ K-50
K-54-0030

The Toxicity of the Vapor
of Aroclors 1242 and 1254

K-50
K-54-0030

by

Joseph F. Treon, Ph.D.
Frank P. Cleveland, M.D.
John Cappel, B.S.
Frederic E. Shaffer, M.S.
Ralph W. Atchley, B.S.
John P. Torbeck, B.S.



June 22, 1955

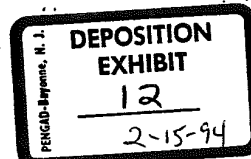
THE KETTERING LABORATORY

in the

Department of Preventive Medicine and Industrial Health

College of Medicine

UNIVERSITY OF CINCINNATI, CINCINNATI, OHIO



NEV 016552

Sequence of Tables

1. The Mortality, Length of Survival, and Changes in Weight of Animals Exposed to the Vapor of Aroclor 1242 in Air. Experiment No. 1.
2. The Mortality, Length of Survival, and Changes in Weight of Animals Exposed to the Vapor of Aroclor 1242 in Air. Experiment No. 2.
3. The Mortality, Length of Survival, and Changes in Weight of Animals Exposed to the Vapor of Aroclor 1254 in Air. Experiment No. 1.
4. The Mortality, Length of Survival, and Changes in Weight of Animals Exposed to Conditioned Air Containing No Vapor of Aroclor 1242 or Aroclor 1254. Experiment No. 1.
5. Summary of the Data on the Mortality Among Groups of Experimental and Control Animals.
6. The Average Changes in Weight of Experimental and Control Animals.
7. The Relationships of the Weights of Certain Organs to the Body Weights of Animals Exposed to the Vapor of Aroclor 1242 or Aroclor 1254.
8. Summary of the Data on the Relationship of the Weight of the Liver or the Kidneys to the Body Weight of Animals Exposed to the Vapor of Aroclor 1242 or Aroclor 1254.
9. The "Prothrombin Time" (Method of Kato) of the Blood of Animals Following Their Exposure to the Vapor of Aroclor 1242 or Aroclor 1254 in Air.
10. The "Prothrombin Time" (Method of Kato) of the Blood of Animals Following Their Confinement in Cages Supplied with Conditioned Air Containing No Vapor of Aroclor 1242 or Aroclor 1254.
11. The Effect of the Exposure of Cats and Rabbits to the Vapor of Aroclor 1242 or Aroclor 1254 in Air for 7 Hours per Day on 5 Days per Week Over a Period of Several Months, Upon the Apparent Prothrombin Content (Method of Kato) of Their Blood.

NEV 016553

12. The Effects of the Intermittent Inhalation of the Vapor of Aroclor 1242 or Aroclor 1254, or of Conditioned Air, upon the Numbers of Erythrocytes and Leucocytes and upon the Concentration of Hemoglobin in the Peripheral Blood of Animals.
13. The Average Numbers of Erythrocytes and Leucocytes and the Average Concentration of Hemoglobin in the Peripheral Blood of Experimental and Control Animals.
14. Application of the "t" Test to the Hematological Data Obtained in Observations on Exposed and Control Animals.

NEV 016554

Sequence of Figures

- A. Equipment for Volatilizing Aroclor, Chamber and Equipment for Combustion and Collection of Samples.
- B. Front View of 600-Liter Chamber.
- C. Humidifier for Furnace Sampling Towers.
- D. Sampling Towers.
- E. Standard Curve for the Determination of Aroclor 1242 and Aroclor 1254.
- 1. Weight of Cat A-446 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1242 in Air - 8.6 Micrograms per Liter (Experiment No. 1).
- 2. Weights of Individual Guinea Pigs B-331 to B-336 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1242 in Air - 8.6 Micrograms per Liter (Experiment No. 1).
- 3. Average and Range of Weight of Guinea Pigs B-331 to B-336 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1242 in Air - 8.6 Micrograms per Liter (Experiment No. 1).
- 4. Average and Range of Weight of Mice B-251 to B-260 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1242 in Air - 8.6 Micrograms per Liter (Experiment No. 1).
- 5. Weights of Individual Rats L-436 to L-440 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1242 in Air - 8.6 Micrograms per Liter (Experiment No. 1).
- 6. Weights of Individual Rats L-441 to L-445 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1242 in Air - 8.6 Micrograms per Liter (Experiment No. 1).

NEV 016555

7. Average and Range of Weight of Rats L-436 to L-445 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1242 in Air - 8.6 Micrograms per Liter (Experiment No. 1).
8. Weights of Individual Rabbits I-374 to I-377 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1242 in Air - 8.6 Micrograms per Liter (Experiment No. 1).
9. Average and Range of Weight of Rabbits I-374 to I-377 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1242 in Air - 8.6 Micrograms per Liter (Experiment No. 1).
10. Weight of Cat A-445 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1242 in Air - 6.8 Micrograms per Liter (Experiment No. 2).
11. Weights of Individual Guinea Pigs B-325 to B-328 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1242 in Air - 6.8 Micrograms per Liter (Experiment No. 2).
12. Weights of Individual Guinea Pigs B-329, B-330 and B-419 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1242 in Air - 6.8 Micrograms per Liter (Experiment No. 2).
13. Average and Range of Weight of Guinea Pigs B-325 to B-330 and B-419 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1242 in Air - 6.8 Micrograms per Liter (Experiment No. 2).
14. Average and Range of Weight of Mice B-241 to B-250, B-376 and B-464 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1242 in Air - 6.8 Micrograms per Liter (Experiment No. 2).
15. Weights of Individual Rats L-526 to L-530 Recorded at Semi-weekly intervals During Exposure to the Vapor of Aroclor 1242 in Air - 6.8 Micrograms per Liter (Experiment No. 2).

NEV 016556

16. Weights of Individual Rats L-531 to L-535 and L-836 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1242 in Air - 6.8 Micrograms per Liter (Experiment No. 2).
17. Average and Range of Weight of Rats L-526 to L-535 and L-836 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1242 in Air - 6.8 Micrograms per Liter (Experiment No. 2).
18. Weights of Individual Rabbits I-370 to I-373 and I-964 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1242 in Air - 6.8 Micrograms per Liter (Experiment No. 2).
19. Average and Range of Weight of Rabbits I-370 to I-373 and I-964 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1242 in Air - 6.8 Micrograms per Liter (Experiment No. 2).
20. Weight of Cat A-444 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1254 in Air - 5.4 Micrograms per Liter (Experiment No. 1).
21. Weights of Individual Guinea Pigs B-319 to B-322 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1254 in Air - 5.4 Micrograms per Liter (Experiment No. 1).
22. Weights of Individual Guinea Pigs B-323, B-324, B-415 and B-418 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1254 in Air - 5.4 Micrograms per Liter (Experiment No. 1).
23. Average and Range of Weight of Guinea Pigs B-319 to B-324, B-415 and B-418 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1254 in Air - 5.4 Micrograms per Liter (Experiment No. 1).
24. Average and Range of Weight of Mice B-231 to B-240 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1254 in Air - 5.4 Micrograms per Liter (Experiment No. 1).

NEV 016557

25. Weights of Individual Rats L-299 and L-517 to L-520 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1254 in Air - 5.4 Micrograms per Liter (Experiment No. 1).
26. Weights of Individual Rats L-521 to L-525 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1254 in Air - 5.4 Micrograms per Liter (Experiment No. 1).
27. Average and Range of Weight of Rats L-299 and L-517 to L-525 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1254 in Air - 5.4 Micrograms per Liter (Experiment No. 1).
28. Weights of Individual Rabbits I-362 to I-364 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1254 in Air - 5.4 Micrograms per Liter (Experiment No. 1).
29. Weights of Individual Rabbits I-453, I-587, I-718 and I-887 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1254 in Air - 5.4 Micrograms per Liter (Experiment No. 1).
30. Average and Range of Weight of Rabbits I-362 to I-364, I-453, I-587, I-718 and I-887 Recorded at Semi-weekly Intervals During Exposure to the Vapor of Aroclor 1254 in Air - 5.4 Micrograms per Liter (Experiment No. 1).
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The Toxicity of the Vapor of Aroclors 1242 and 1254

Scope

The purpose of this investigation was to determine the effects upon animals of various species, of moderately prolonged intermittent exposure to air bearing the vapor of either Aroclor 1242 or Aroclor 1254. This report deals with the results obtained in experiments in which, for periods ranging up to 4 months, animals were subjected to respiratory exposure to air containing the Aroclors in concentrations that approached saturation and so were greater than any likely to be encountered in industry. The animals failed to exhibit any signs of intoxication during or after the exposure to which they were subjected, and the numbers of deaths which occurred among the exposed animals were comparable to those which resulted from incidental causes in the control groups. Gross and microscopic examination of the tissues of the animals of both the exposed and the control groups disclosed the existence of certain abnormalities which did not differ significantly in their severity or frequency of occurrence among the test and control groups. Other experiments which involve more prolonged exposure on the part of animals to lower concentrations of these materials in air, are now in progress.

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Summary of Results

1. No signs of intoxication were observed in any of the numbers of a group of animals, all of which survived throughout a period of 24 days, on each of 17 of which they were subjected to the inhalation of air bearing 8.6 micrograms of Aroclor 1242 per liter (0.83 ppm) for 7 hours. The viscera of representative animals that were examined microscopically were normal.

2. The incidence of mortality among the animals of all species, excepting the rabbits, in 2 groups that were subjected (7 hours on each of 62 or 83 days over a period of 120 or 121 days) to the inhalation of air bearing, respectively, Aroclor 1242 in the concentration of 6.83 micrograms per liter (0.66 ppm), and Aroclor 1254 in that of 5.40 micrograms per liter (0.41 ppm), was no greater than that among a similarly constituted control group. Two of 5 rabbits died following exposure to the vapor of Aroclor 1242, as did 3 of 7 rabbits exposed to the vapor of Aroclor 1254, whereas only 1 of 5 of the control rabbits died. The survivors, with the exception of the guinea pigs exposed to the vapor of 1254, grew equally as well as did the controls. The average weights of the livers and kidneys (relative to body weights) of the animals exposed to Aroclor 1242, did not differ significantly from those of the

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unexposed group, but the livers of the rats that were exposed to Aroclor 1254 were enlarged. The prothrombin content of the blood of the animals exposed to either Aroclor, as measured by the method of Kato, remained within normal limits.

The deaths that occurred among the test and control animals were the apparent result of an appreciable incidence of pneumonia. The lesions of certain of the animals were those of a frank pneumonia; in others such lesions were not fully developed. Degenerative changes in the viscera were usually found, in varying degrees of severity, in association with the pneumonia, but in certain animals, test and control alike, the degenerative changes were more evident than the pneumonia. The similarity of the lesions in test and control animals, and the lack of characteristic evidence of chemical pneumonitis, lead to the reasonable, but not altogether certain conclusion that all of these fatalities resulted from intercurrent disease among the animals, and not from the effects of their exposure to the Aroclors. For practical purposes, this conclusion is subject to the critique of further experiments involving more prolonged exposure of animals to somewhat lower concentrations.

Properties of the Experimental Materials

Aroclor 1242 is a light straw-colored, mobile liquid with the composition of a chlorinated biphenyl. A letter from

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P.G. Benigus, dated August 30, 1953, states that it contains 42.0 ± 0.5 per cent of chlorine, which corresponds to the presence of 3 chlorine atoms. The positions of the chlorine atoms within the molecule have not been determined. Monsanto Application Bulletin No. P-115, gives its specific gravity at $25^{\circ}/25^{\circ}$ as 1.378 to 1.388, and states that it has a distillation range of 325° to 360°C , a refractive index (D-line at 20°C) of 1.627 to 1.629, a Saybolt Universal viscosity of 100°F of 80 to 93 seconds, and a flash point (Cleveland Open Cup) of 176° - 180°C . Its vapor pressure is about 4 mm at 150°C and about 30 mm at 200°C . Although insoluble in water and glycerine, it is soluble in most organic substances. At 745 mm of mercury and 25°C , the concentration of 1 mg of the vapor of Aroclor 1242 per liter of air is equivalent to 96.9 ppm by volume.

Aroclor 1254, which corresponds to pentachlorobiphenyl, is a light straw-colored, viscous liquid. The positions of the chlorine atoms have not been established. The molecular weight of pentachlorobiphenyl is 326.445, of which 54.3 per cent is chlorine. Benigus (ibid) reported that Aroclor 1254 contained 54.0 ± 0.5 per cent chlorine. Bulletin No. P-115, states that Aroclor 1254 has a specific gravity at $25^{\circ}/25^{\circ}\text{C}$ of 1.538 to 1.548, a distillation range of 365° to 390°C , a refractive index (D-line at 20°C) of 1.639 to 1.641, and a Saybolt Universal

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viscosity at 100°F of 1,800 to 2,500 seconds. The vapor pressure at 150°C is about 1.3 mm and at 200°C is about 9 mm. It is soluble in most organic substances, but it is insoluble in water and glycerine.

At 745 mm of mercury and 25°C the concentration of 1 mg of Aroclor 1254 per liter is equivalent to 76.5 ppm by volume.

Experimental Methods

Exposure to the Vapor. In a preliminary experiment with the vapor of Aroclor 1242, a group of animals, consisting of a cat, 6 guinea pigs, 10 mice, 4 rabbits and 10 rats, were confined for 7 hours on each of 5 days per week in a rectangular plywood chamber (volume 600 liters), of which the inner metal lining was coated with a baked chemically resistant plastic, through which was passed a stream of air laden with Aroclor 1242 in a known concentration. The stream of air, conditioned with respect to temperature ($75 \pm 30^\circ\text{F}$ inside the chamber), dust and humidity, entered the chamber through an "Anemostat," located at the center of its top, at the rate of 500 liters per minute, as measured by an inclined manometer attached to a venturimeter (Figure A). The air was withdrawn by suction from the chamber through an exit tube located on the rear wall near the floor, equidistant from the sides.

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Aroclor 1242 was volatilized from a heated glass well maintained at 132° to 138°C. The air passed over the surface of the liquid before entering the chamber (Figure B).

In a second similar experiment with the same Aroclor, the temperature of the liquid was kept at 100° to 105°C.

While the second experiment with Aroclor 1242 was in progress an experiment involving the vapor of Aroclor 1254 was carried out in a second chamber similar to the first except for the absence of a plastic inner lining. The rate at which air flowed over the liquid Aroclor 1254, which was maintained at 130° - 135°C, was 400 liters per minute.

In order to accustom the animals to the experimental procedure, they were kept in their respective chambers for 7 hours on each of 4 consecutive days during the week prior to the introduction of the Aroclor vapor into the chamber. For purposes of control, a third group of animals was confined throughout a like period before and during the period of the exposure to the Aroclors in another chamber supplied only with conditioned air.

Method for the Determination of Aroclor 1242 or Aroclor 1254 in Air. These materials were determined quantitatively by virtue of the fact that, on thermal decomposition, they yield hydrochloric acid which, with silver nitrate, forms a suspension, the density of which could be measured by means of the Beckman spectrophotometer at 500 mμ.

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On each day, 2 samples of air from each respiratory chamber were collected by passing air at the rate of 1 liter per minute for 15 minutes through a Willson combustion furnace, and then through 2 midget bubblers, in series, each containing 10 ml of 0.1 N sodium hydroxide. A quartz tube (13 mm outside diameter, 7 mm inside diameter and 16.25" in length) contained several strips of folded platinum foil. The middle 9" of the tube was wrapped with a heating unit. The heating unit of the Willson furnace (Figure C) consisted of 20 feet of B. and S. gauge 22 nichrome wire (1 ohm per foot), and was covered with asbestos cement. The sample of air was humidified by a cotton wick saturated with water placed 0.5 inch upstream from the quartz tube. With the furnace maintained at 850°C, the Aroclor was decomposed to free chlorine, which reacted with the moisture to form HCl; this was absorbed in the 0.1 N sodium hydroxide in the midget bubblers (Figure D) equipped with fritted-glass bubblers (Mine Safety Appliance Company No. 43867).

The 0.1 N sodium hydroxide was prepared by dissolving 4.0 g of pellets (ACS specifications 0.01 per cent Cl) in about 50 ml of double distilled water. To this was added 10 g of arsenic trioxide (chloride-free) that had been dissolved in water and filtered through Whatman No. 42 filter paper. The combined solutions were diluted to 1000 milliliters.

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The sample was transferred to a 25 ml graduated cylinder to which 1 drop of a solution of phenolphthalein was added (1 g of phenolphthalein dissolved in 100 ml of CP methanol). The solution was neutralized with 3N nitric acid (190 ml CP concentrated nitric acid diluted to 1000 ml with double-distilled water) and diluted to 23 ml with double-distilled water. One ml of 3N nitric acid was added to obtain a pH of 1. After adding 1 ml of a solution of silver nitrate (3 grams of AgNO_3 diluted to 1000 ml with double-distilled water), the suspension was mixed by inversion. After standing 30 minutes, the transmission of wave-length 500 m μ was determined in a 50 mm cell by means of a Beckman spectrophotometer which was set against a corresponding cell containing a reagent blank.

The amount of Aroclor was determined from a standardized curve obtained in a similar manner from known quantities of sodium chloride. A curve relating the transmission at 500 m μ of a suspension resulting from sodium chloride over the range of 5.0 to 120 micrograms per 25 ml of final suspension is shown in Figure E. On the basis of 42.0 per cent of chlorine in Aroclor 1242 and 54.0 per cent in Aroclor 1254, 1 microgram of sodium chloride is equivalent to 1.4442 micrograms of Aroclor 1242 or to 1.1026 micrograms of Aroclor 1254.

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Experimental Results

Aroclor 1242, Experiment No. 1. One cat, 6 guinea pigs, 10 mice, 4 rabbits and 10 rats survived when subjected for 7 hours on each of 17 days over the period of 24 days to the inhalation of air bearing the vapor of Aroclor 1242 in the concentration of 8.6 micrograms per liter (0.83 ppm, Tables 1 and 5). No signs of intoxication were observed.

The weights of the individual animals (cat, guinea pigs, rats and rabbits) have been plotted in Figures 1, 2, 5, 6 and 8. The averages and the ranges of the weights of guinea pigs, mice, rats and rabbits have been plotted at semi-weekly intervals in Figures 3, 4, 7 and 9, respectively.

Despite slight fluctuations in their weight during the period of exposure, all of the animals, except the guinea pigs, gained in weight during the period of exposure (Table 6). The average gains made by the various species, expressed as percentage of the initial weights were: cats, 8.1; mice, 10.3; rabbits, 7.7; and rats, 2.1. The loss in weight on the part of the guinea pigs averaged 2.0 per cent; most of them were gaining weight (Figure 2) at the termination of the period of exposure.

All of the animals were examined post-mortem, and since gross examination of the viscera did not reveal any significant alterations, the tissues of only representative animals

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(1 cat, 4 guinea pigs, 6 mice, 4 rabbits and 6 rats) were sectioned and examined microscopically. No abnormalities were found in the viscera of these animals.

Aroclor 1242, Experiment No. 2 (6.83 γ)/1). The pertinent data relating to the fate of the individual animals in the Experiment No. 2 are presented in Table 2 and summarized in Table 5. This group consisted originally of 1 cat, 6 guinea pigs, 10 mice, 4 rabbits and 10 rats, but a few additional animals were used to replace those which died. One cat, 4 guinea pigs, 6 mice, 2 rabbits and 8 rats survived throughout their exposure, for 7 hours on each of 82 days over a period of 120 days, to air bearing the vapor of Aroclor 1242 in the concentration of 6.83 micrograms per liter (0.66 ppm). Certain animals (2 guinea pigs, 1 mouse, 1 rabbit and 1 rat) were killed after 65 to 68 periods of exposure for examination. In addition, 1 rat and 2 mice survived throughout 23, 41 and 58 periods of exposure, respectively. Seven fatalities occurred as follows: 1 guinea pig after 15 periods of exposure; 3 mice after 24, 41 and 70 periods of exposure, respectively; 2 rabbits after 15 and 64 periods of exposure, respectively; and 1 rat after 56 periods of exposure.

Except in the case of the rabbits, the incidence of mortality among the controls was equally as great (or greater, Tables 4 and 5) as that among the experimental animals. One cat, 3

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mice, 3 guinea pigs, 3 rabbits and 8 rats survived, following their confinement, for 7 hours on each of 84 days over a period of 122 days, in a chamber in which the air, conditioned with respect to dust, humidity and temperature, contained no vapor of either Aroclor. One guinea pig, 1 mouse and 1 rat were killed after 66 (or 68) periods of exposure. Other animals introduced later in the experiment survived throughout the following numbers of periods of exposure, respectively; 2 guinea pigs, 20 and 77; 3 mice, 14, 14 and 19; 1 rabbit, 19; and 1 rat, 44. Eleven fatalities occurred at stated times among the control animals, as follows: 2 guinea pigs, after 7 and 60 periods, respectively; 7 mice after 5, 59, 68, 69, 70, 72 and 82 periods, respectively; and 1 rat and 1 rabbit after 42 and 65 periods, respectively.

No uniform or suggestive signs of intoxication were observed among either the experimental or control groups.

The weights of the individual animals (cat, guinea pigs, rats and rabbits) exposed to Aroclor 1242 are shown in Figures 10, 11, 12, 15, 16 and 18. The average weight, and the greatest and least weights attained by any individual within the groups of guinea pigs, mice, rats and rabbits, are plotted in Figures 13, 14, 17 and 19, respectively. Comparable data on the controls are given in Figures 31 to 41.

The surviving experimental animals grew equally as well (or better) as the controls (Table 6). During the period of exposure to Aroclor 1242 (6.83 γ /1), the average gains, expressed as percentages of the initial weights, by the various animals were as follows: cat, 22.2; guinea pigs, 56.4; mice 21.2; rabbits, 50.5; and rats, 17.6. The comparable findings on the controls were: cat, 6.6; guinea pigs, 56.6; mice, 17.4; rabbits, 44.5; and rats, 16.6.

The weights of the livers and kidneys and the relationships of their weights to the body weights (expressed as grams per 100 grams of body weight) of the individual animals that survived following exposure to the vapor of Aroclor 1242, are shown in Table 7, which also gives the corresponding data on the controls. Comparison by the "F" test of the variances of the ratios of the liver or kidneys to the body weights of rats, guinea pigs and rabbits exposed to the vapor of Aroclor 1242 (6.83 γ /1), with those of the controls, revealed no significant differences ($P > 0.05$) except in the case of the livers of the rats. Application of the "t" test to the mean values (Table 6) revealed no significant differences between the test and control groups of guinea pigs, rats and rabbits.

The apparent prothrombin content of the blood, as measured by the method of Kato (Am. J. Clin. Path., 10:147, 1940), of

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certain animals that had been exposed to the vapor of Aroclor 1242, are given in Table 9. Comparable findings on the controls are presented in Table 10. In Table 12 the percentile relationships of the clotting power have been obtained arbitrarily by dividing 100 times the average clotting time of the blood of the control animals by the clotting time of the blood of the experimental animals on the same day. No diminution in the clotting power of the blood, as a measure of the impairment of the function of the liver, was induced by the exposure of the animals to the vapor of Aroclor 1242 when present in the air in the concentration of 6.8 micrograms per liter.

Determinations of the numbers of erythrocytes and leucocytes and of the hemoglobin content in the peripheral blood of individual guinea pigs and rabbits subjected to inhalation of the vapor of Aroclor 1242 in the concentration of 6.8 micrograms per liter, are given in Table 12, which also includes comparable results on control animals. These values have been summarized in Table 13. Application of the "t" test to differences in the mean values (Table 14) for the experimental and control animals yielded border-line evidence of significant differences in the numbers of leucocytes and in the concentration in the hemoglobin in the blood of the guinea pigs, but these were small and of opposite sign (the leucocytes in the blood of the test animals

were low in number while the hemoglobin content was high), as well as being subject to individual variation, and they cannot be regarded as of physiological significance. Of the 7 animals that died during the second experiment with Aroclor 1242, all but 2 mice were examined post-mortem. The tissues of 2 mice had undergone significant changes, post-mortem, and were not examined microscopically; gross evidence to determine the cause of death was not available. A third mouse (B-244) was examined and found to have a malignant lymphoma that involved the liver, spleen, kidneys and heart, and therefore its death was attributed to natural cause. The cause of the death of the guinea pig was uncertain, but the 2 rabbits and the rat were found to have died of pneumonia, and not, apparently, of chemical pneumonitis.

Generally, the survivors among the various species had normal viscera. However, 2 guinea pigs and 1 rat had degenerative lesions in the liver and kidneys, 1 guinea pig had acute lobular pneumonia, and 1 rat had interstitial pneumonia.

Eleven of the animals (2 guinea pigs, 7 mice, 1 rabbit and 1 rat), that had been confined in conditioned air only, died. The viscera of 6 of these (1 guinea pig, 4 mice and 1 rabbit) were examined microscopically. Each of the animals examined had died with pneumonia, in association with degenerative

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changes of varying severity in the brain, liver and kidneys. Animals of all species that survived were examined and found to have normal viscera, but for scattered pneumonic lesions in certain of the guinea pigs and mice, and for one case of cellulitis in a rabbit.

Aroclor 1254, Experiment No. 1 (5.40 μ /l). The pertinent data relating to the change in weight and the fate of each individual animal during and following prolonged intermittent exposure to the vapor of Aroclor 1254 are given in Table 3 and are summarized in Table 5. The original group consisted of the same numbers and types of animals employed in the other experiments. One cat, 3 guinea pigs, 4 mice, 2 rabbits, and 9 rats survived throughout their exposure, for 7 hours on each of 83 days over a period of 121 days, to air containing Aroclor 1254 in the concentration of 5.40 micrograms per liter (0.41 ppm). Certain other animals (3 guinea pigs, 6 mice, 1 rabbit and 1 rat) were killed for examination after 66 or more (up to 74) periods of exposure. One rabbit, introduced after the experiment had been under way for some time, survived, having undergone 33 periods of exposure. Five animals died during the experiment, 2 guinea pigs after 12 and 17 periods of exposure, respectively, and 3 rabbits after 15, 15 and 20 periods of exposure. Except for the rabbits, the incidence of mortality among the exposed and control groups was comparable (Tables 4 and 5). No signs of intoxication were noted among the experimental animals.

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The weights of the individual animals (cat, guinea pigs, rats and rabbits) are given in Figures 20, 21, 22, 25, 26, 28 and 29, while the average weights of the guinea pigs, mice, rats and rabbits are shown in Figures 23, 24, 27 and 30, respectively.

Except in the case of the guinea pigs and rats, the animals that survived throughout the period of exposure to the vapor of Aroclor 1254 grew equally as well as the controls (Table 6). During the period of exposure the gains made by the animals of various species, expressed as percentages of their initial weights, were as follows: cat, 22.1; guinea pigs, 40.7; mice, 28.9; rabbits, 39.7; and rats, 6.9. The lesser gain by the experimental rats may be explained by the fact that they were initially somewhat heavier. The corresponding values for the control animals have been given previously and are also shown in Table 6.

The weights of the livers and kidneys, and the ratios of these to the body weights, of the animals that survived throughout the exposure are shown in Table 7. Application of the "t" test to the differences in the mean values which characterized the experimental and control animals (Table 8) shows that the weights of the livers of the exposed rats were significantly greater than those of the controls, the ratio of the liver to the body weight of the former being 5.34 g per 100 g of body weight, that of the latter being 4.15 g/100 grams.

The results on the apparent prothrombin content of the blood of rabbits and of a cat, following exposure to the vapor of Aroclor 1254 (5.40 $\sqrt{1}$) are given in Table 9. Little or no diminution in the clotting power was induced by exposure to the vapor of Aroclor 1254 (Table 11).

The numbers of the cellular elements and the concentration of the hemoglobin in the peripheral blood of the individual animals subjected to inhalation of the vapor of Aroclor 1254 are shown in Table 12 and are summarized in Table 13. The slight apparent increases in the hemoglobin of the guinea pigs and in the numbers of erythrocytes of the rabbits, while statistically significant (Table 14), cannot be taken seriously as expressions of the effects of the inhalation of Aroclor 1254.

The viscera of 4 of the 5 animals (2 guinea pigs and 3 rabbits) that died after varying periods of exposure to the vapor of Aroclor 1254, exhibited diffuse degenerative changes in the brain, liver and kidneys, in association with pneumonia. Animals that survived, following exposure to the Aroclor, were examined post-mortem, and the viscera from representative animals were examined microscopically. The viscera of the cat and guinea pigs were normal. All of the mice, except one, had normal viscera; the exception (B-237) had slight degenerative lesions in the liver. A rabbit killed 1 day after the 66th

period of exposure, had degeneration of the liver and kidneys, and pulmonary hyperemia and edema. Otherwise, the rabbits that were examined had essentially normal viscera. All of the rats that were examined had degenerative changes in the liver, and in 5 of the 10, these changes were found to be associated with pneumonia of low grade.

The Aroclors did not appear to act as primary pulmonary irritants. The pneumonia that was observed in both the experimental and control animals had the characteristics of an infectious rather than a chemical process. The toxic degenerative changes which were found in the liver and kidneys of all species of animals, in both experimental and control groups, while not associated with pneumonia in all instances, may well have owed their presence to the intercurrent disease which was characterized, chiefly, in most instances as pneumonia.

Discussion

The probability of the occurrence of the concentrations of vapor maintained in these experiments in actual industrial practice is remote, since these values approach those of atmosphere saturated with the Aroclors. (The significance of particulate matter, in the form of condensed droplets or of products of thermal decomposition, has not been involved in these experiments.)

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Since the animals which were killed for examination after 65 to 68 periods of exposure were found to have degenerative lesions in their viscera which could not, with certainty, be attributed to extra-experimental causes, these experiments were terminated and others have been initiated at about 1/3 to 1/4 the concentration.

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Project No. 47.

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Table 1
The Mortality, Length of Survival, and Changes in Weight of
Animals Exposed to the Vapor of Aroclor 1242 in Air

(Experiment No. 1)

Concentration ppm	Duration of Exposure (hours)	Identification Number	Initial Weight (kg)	Change in Weight Expressed as Percentage of Initial Weight		Fate	Length of Sur- vival After Final Period of Exposure
				During Period of Exposure	After Period of Exposure		
8.6	17 x 7.0	Cat A-446	2.863	+ 8.1	+ 0.2	Survived	Killed 17 days
	17 x 7.0	Guinea Pig B-331	0.593	- 10.4	- 1.2	Survived	Killed 17 days
	17 x 7.0	Guinea Pig B-332	0.546	- 8.4	- 3.7	Survived	Killed 17 days
	17 x 7.0	Guinea Pig B-333	0.860	+ 3.8	- 2.0	Survived	Killed 3 days
	17 x 7.0	Guinea Pig B-334	0.742	+ 8.4	- 2.8	Survived	Killed 3 days
	17 x 7.0	Guinea Pig B-335	0.463	- 0.4	- 1.5	Survived	Killed 10 days
	17 x 7.0	Guinea Pig B-336	0.685	- 5.0	+ 1.9	Survived	Killed 10 days
	17 x 7.0	Mouse B-251	0.026	+ 3.8	+ 3.8	Survived	Killed 10 days
	17 x 7.0	Mouse B-252	0.028	+ 3.6	+ 3.6	Survived	Killed 17 days
	17 x 7.0	Mouse B-253	0.027	+ 0.0	+ 0.0	Survived	Killed 17 days
	17 x 7.0	Mouse B-254	0.026	+ 7.7	+ 3.8	Survived	Killed 17 days
	17 x 7.0	Mouse B-255	0.021	+ 19.0	+ 4.8	Survived	Killed 3 days
	17 x 7.0	Mouse B-256	0.021	+ 19.0	+ 14.3	Survived	Killed 3 days
	17 x 7.0	Mouse B-257	0.021	+ 14.3	+ 0.0	Survived	Killed 10 days
	17 x 7.0	Mouse B-258	0.028	+ 8.1	+ 0.0	Survived	Killed 10 days
	17 x 7.0	Mouse B-259	0.019	+ 10.5	+ 5.3	Survived	Killed 17 days
	17 x 7.0	Mouse B-260	0.023	+ 21.7	+ 17.4	Survived	Killed 17 days
	17 x 7.0	Rabbit I-374	3.800	+ 9.0	+ 13.9	Survived	Killed 3 days
	17 x 7.0	Rabbit I-375	4.180	+ 8.4	+ 5.8	Survived	Killed 10 days

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Concentration 7/1	ppm	Duration of Exposure (hours)	Identification Number	Initial Weight (kg)	Change in Weight Expressed as Percentage of Initial Weight		Fate	Length of Sur- vival After Final Period of Exposure
					During Period of Exposure	After Period of Exposure		
8.6	0.83	17 x 7.0	Rabbit I-376	4.225	+ 2.9	+ 1.8	Survived	Killed 17 days
		17 x 7.0	Rabbit I-377	3.400	+ 10.3	+ 6.8	Survived	Killed 24 days
		17 x 7.0	Rat I-436	0.227	+ 1.8	+ 2.6	Survived	Killed 3 days
		17 x 7.0	Rat I-437	0.250	+ 4.4	+ 0.8	Survived	Killed 3 days
		17 x 7.0	Rat I-438	0.254	+ 1.2	+ 0.4	Survived	Killed 10 days
		17 x 7.0	Rat I-439	0.207	+ 0.5	+ 6.3	Survived	Killed 10 days
		17 x 7.0	Rat I-440	0.228	+ 2.2	+ 0.4	Survived	Killed 17 days
		17 x 7.0	Rat I-441	0.250	+ 0.8	+ 5.6	Survived	Killed 10 days
		17 x 7.0	Rat I-442	0.240	+ 4.6	+ 1.7	Survived	Killed 17 days
		17 x 7.0	Rat I-443	0.230	+ 2.2	+ 0.4	Survived	Killed 17 days
		17 x 7.0	Rat I-444	0.238	+ 1.3	+ 3.8	Survived	Killed 17 days
		17 x 7.0	Rat I-445	0.254	+ 2.0	+ 5.9	Survived	Killed 17 days

The Mortality, Length of Survival, and Changes in Weight of Animals Exposed to the Vapor of Aroclor 1242 in Air

(Experiment No. 2)

Concentration mg/l	ppm	Duration of Exposure (hours)	Identification Number	Initial Weight (kg)	Change in Weight Expressed as Percentage of Initial Weight		Fate	Length of Sur- vival After Final Period of Exposure
					During Period of Exposure	After Period of Exposure		
6.83	0.66	82 x 7.0	Cat A-445	2.271	+ 22.2	-	Survived	Killed 1 day
		82 x 7.0	Guinea Pig B-325	0.447	+ 43.0	-	Survived	Killed 1 day
		15 x 7.0	Guinea Pig B-326	0.305	- 16.6	-	Died	Killed 1 day
		82 x 7.0	Guinea Pig B-327	0.386	+ 106.7	-	Survived	Killed 1 day
		82 x 7.0	Guinea Pig B-328	0.344	+ 57.0	+ 20.1	Survived	Killed 15 days
		66 x 7.0	Guinea Pig B-329	0.379	+ 8.7	-	Survived	Killed 1 day
		82 x 7.0	Guinea Pig B-330	0.483	+ 45.3	- 0.8	Survived	Killed 15 days
		67 x 7.0	Guinea Pig B-419	0.384	+ 77.9	-	Survived	Killed 1 day
		82 x 7.0	Mouse B-241	0.020	+ 50.0	-	Survived	Killed 1 day
		70 x 7.0	Mouse B-242	0.024	- 12.5	-	Died	Killed 1 day
		66 x 7.0	Mouse B-243	0.023	+ 4.3	-	Survived	Killed 1 day
		24 x 7.0	Mouse B-244	0.017	- 23.5	-	Died	Killed 1 day
		82 x 7.0	Mouse B-245	0.019	+ 31.6	-	Survived	Killed 1 day
		82 x 7.0	Mouse B-246	0.020	+ 10.0	-	Survived	Killed 1 day
		82 x 7.0	Mouse B-247	0.021	+ 14.3	+ 9.5	Survived	Killed 15 days
		41 x 7.0	Mouse B-248	0.019	+ 15.8	-	Died	Killed 15 days

Table 2 - Page 2

Concentration ppm	Duration of Exposure (hours)	Identification Number	Initial Weight (kg)	Change in Weight Expressed as Percentage of Initial Weight		Fate	Length of Sur- vival After Final Period of Exposure
				During Period of Exposure	After Period of Exposure		
6.83	0.66	82 x 7.0 82 x 7.0 58 x 7.0 41 x 7.0	0.020 0.020 0.024 0.024	+ 35.0 + 25.0 + 25.0 - 4.2	+ 5.0 0.0 - -	Survived Survived Survived Survived	Killed 15 days Killed 15 days Killed 1 day Killed 1 day
		82 x 7.0 82 x 7.0 64 x 7.0 66 x 7.0 15 x 7.0	2.672 2.600 2.585 2.490 2.758	+ 59.6 + 51.0 + 20.5 + 40.8 + 2.2	+ 14.4 - - - -	Survived Survived Died Survived Died	Killed 1 day Killed 17 days Killed 1 day Killed 1 day Killed 15th per- iod of exposure
		65 x 7.0 82 x 7.0 82 x 7.0 82 x 7.0 56 x 7.0 82 x 7.0 82 x 7.0 82 x 7.0 82 x 7.0 23 x 7.0	0.220 0.219 0.200 0.202 0.213 0.193 0.206 0.219 0.227 0.228 0.207	+ 9.1 + 15.5 + 27.0 + 15.8 - 6.1 + 14.0 + 14.1 + 19.6 + 17.2 + 36.4 + 6.8	- - - - - - - 3.2 4.8 4.8 -	Survived Survived Survived Survived Died Survived Survived Survived Survived Survived Survived	Killed 1 day Killed 1 day Killed 1 day Killed 1 day Killed 1 day Killed 8 days Killed 15 days Killed 15 days Killed 15 days Killed 1 day

Table 3

The Mortality, Length of Survival, and Changes in Weight of
Animals Exposed to the Vapor of Aroclor 1254 in Air
(Experiment No. 1)

Concentration ppm	Duration of Exposure (hours)	Identification Number	Initial Weight (kg)	Change in Weight Expressed as Percentage of Initial Weight		Fate	Length of Sur- vival After Final Period of Exposure
				During Period of Exposure	After Period of Exposure		
5.40	0.41	83 x 7.0	2.320	+ 22.1	-	Survived	Killed 1 day
		83 x 7.0	0.442	+ 24.7	+ 3.4	Survived	Killed 16 days
		83 x 7.0	0.402	+ 47.3	+ 11.9	Survived	Killed 16 days
		83 x 7.0	0.400	+ 70.5	-	Survived	Killed 1 day
		15 x 7.0	0.360	- 41.1	-	Died	During 15th per- iod of exposure
		12 x 7.0	0.444	- 23.0	-	Died	Killed 1 day
		66 x 7.0	0.388	+ 31.4	-	Survived	Killed 1 day
		71 x 7.0	0.437	+ 5.7	-	Survived	Killed 1 day
		68 x 7.0	0.302	+ 64.4	-	Survived	Killed 1 day
		74 x 7.0	0.022	+ 36.4	-	Survived	Killed 1 day
		74 x 7.0	0.020	+ 35.0	-	Survived	Killed 1 day
		83 x 7.0	0.024	+ 37.5	-	Survived	Killed 1 day
		74 x 7.0	0.019	+ 31.6	-	Survived	Killed 1 day
		74 x 7.0	0.020	+ 30.0	-	Survived	Killed 1 day
		83 x 7.0	0.019	+ 26.3	-	Survived	Killed 1 day
		66 x 7.0	0.020	+ 35.0	-	Survived	Killed 1 day
		74 x 7.0	0.019	+ 21.1	-	Survived	Killed 1 day
		83 x 7.0	0.022	+ 9.1	- 4.5	Survived	Killed 13 days
		Cat A-444					
		Guinea Pig B-319					
		Guinea Pig B-320					
		Guinea Pig B-321					
		Guinea Pig B-322					
		Guinea Pig B-323					
		Guinea Pig B-324					
		Guinea Pig B-415					
		Guinea Pig B-418					
		Mouse B-231					
		Mouse B-232					
		Mouse B-233					
		Mouse B-234					
		Mouse B-235					
		Mouse B-236					
		Mouse B-237					
		Mouse B-238					
		Mouse B-239					

Table 3 - Page 2

Concentration 7/1	ppm	Duration of Exposure (hours)	Identification Number	Initial Weight (kg)	Change in Weight Expressed as Percentage of Initial Weight		Fate	Length of Sur- vival After Final Period of Exposure
					During Period of Exposure	After Period of Exposure		
5.40	0.41	83 x 7.0	Mouse B-210	0.022	+ 27.3	0.0	Survived	Killed 15 days
		66 x 7.0	Rabbit I-362	2.875	+ 37.8	-	Survived	Killed 1 day
		83 x 7.0	Rabbit I-363	2.818	+ 46.1	-	Survived	Killed 1 day
		83 x 7.0	Rabbit I-364	2.993	+ 38.3	+ 1.6	Survived	Killed 16 days
		15 x 7.0	Rabbit I-453	2.324	- 41.3	-	Died	2 days
		15 x 7.0	Rabbit I-587	2.654	- 8.3	-	Died	3 days
		20 x 7.0	Rabbit I-718	2.729	- 2.6	-	Died	1 day
		33 x 7.0	Rabbit I-887	2.100	+ 36.7	-	Survived	Killed 1 day
		83 x 7.0	Rat L-299	0.224	+ 7.6	-	Survived	Killed 1 day
		66 x 7.0	Rat L-517	0.235	- 6.4	-	Survived	Killed 1 day
		83 x 7.0	Rat L-518	0.217	- 1.4	-	Survived	Killed 1 day
		83 x 7.0	Rat L-519	0.224	+ 24.1	-	Survived	Killed 1 day
		83 x 7.0	Rat L-520	0.237	+ 2.1	-	Survived	Killed 1 day
		83 x 7.0	Rat L-521	0.231	- 1.3	-	Survived	Killed 15 days
		83 x 7.0	Rat L-522	0.235	+ 10.6	-	Survived	Killed 15 days
		83 x 7.0	Rat L-523	0.226	+ 4.0	- 1.3	Survived	Killed 15 days
		83 x 7.0	Rat L-524	0.242	+ 12.8	- 0.8	Survived	Killed 15 days
		83 x 7.0	Rat L-525	0.200	+ 13.5	+ 3.0	Survived	Killed 15 days

Table 4
The Mortality, Length of Survival, and Changes in Weight of
Animals Exposed to Conditioned Air Containing No Vapor of
of Aroclor 1242 or Aroclor 1254

(Experiment No. 1)

Duration of Exposure (hours)(1)	Identification Number	Initial Weight (kg)(1)	Change in Weight Expressed as Percentage of Initial Weight		Fate	Length of Survival After Final Period of Exposure Unless Otherwise Stated
			During Period of Exposure (1)	After Period of Exposure		
84 x 7.0	Cat A-435	2.308	+ 6.6	-	Survived	Killed 1 day
84 x 7.0	Guinea Pig B-313	0.335	+ 95.2	-	Survived	Killed 1 day
66 x 7.0	Guinea Pig B-314	0.413	+ 25.7	-	Survived	Killed 1 day
84 x 7.0	Guinea Pig B-315	0.380	+ 70.7	+ 15.5	Survived	Killed 15 days
7 x 7.0	Guinea Pig B-316	0.384	- 6.3	-	Died	Killed 1 day
84 x 7.0	Guinea Pig B-317	0.454	+ 77.1	+ 14.5	Survived	Killed 15 days
60 x 7.0	Guinea Pig B-318	0.414	+ 20.3	-	Died	Killed 60th period of exposure
77 x 7.0	Guinea Pig B-402	0.469	+ 33.5	-	Survived	Killed 1 day
20 x 7.0	Guinea Pig B-576	0.455	+ 29.2	-	Survived	Killed 1 day
84 x 7.0	Mouse B-221	0.021	+ 14.3	- 14.3	Survived	Killed 15 days
68 x 7.0	Mouse B-222	0.021	+ 23.8	-	Survived	Killed 1 day
68 x 7.0	Mouse B-223	0.022	+ 9.1	-	Died	1 day
72 x 7.0	Mouse B-224	0.021	0.0	-	Died	During 72nd period of exposure
82 x 7.0	Mouse B-225	0.021	0.0	-	Died	During 82nd period of exposure

Table 4 - Page 2

Duration of Exposure (hours)(1)	Identification Number	Initial Weight (kg)(1)	Change in Weight Expressed as Percentage of Initial Weight		Fate	Length of Survival After Initial Period of Exposure Unless Otherwise Stated
			During Period of Exposure(1)	After Period of Exposure		
69 x 7.0	Mouse B-226	0.021	0.0	-	Died	3 days
59 x 7.0	Mouse B-227	0.022	40.9	-	Died	1 day
70 x 7.0	Mouse B-228	0.022	4.5	-	Died	During 70th period of exposure
84 x 7.0	Mouse B-229	0.023	30.4	0.0	Survived	Killed 3 days
84 x 7.0	Mouse B-230	0.023	0.0	0.0	Survived	Killed 3 days
5 x 7.0	Mouse B-499	0.023	-	-	Died	3 days
14 x 7.0	Mouse B-531	0.023	4.3	0.0	Survived	Killed 3 days
14 x 7.0	Mouse B-532	0.013	84.6	0.0	Survived	Killed 3 days
19 x 7.0	Mouse B-533	0.030	26.7	0.0	Survived	Killed 3 days
84 x 7.0	Rabbit I-365	3.084	46.1	-	Survived	Killed 1 day
84 x 7.0	Rabbit I-366	3.162	48.6	-	Survived	Killed 1 day
84 x 7.0	Rabbit I-368	2.568	50.7	-18.0	Survived	Killed 15 days
65 x 7.0	Rabbit I-369	3.352	44.0	-	Died	During 65th period of exposure
19 x 7.0	Rabbit I-795	2.243	32.5	-	Survived	Killed 1 day
84 x 7.0	Rat L-507	0.209	14.8	-1.4	Survived	Killed 15 days
84 x 7.0	Rat L-508	0.231	2.2	+3.9	Survived	Killed 15 days
84 x 7.0	Rat L-509	0.212	9.9	+3.8	Survived	Killed 15 days
84 x 7.0	Rat L-510	0.225	19.1	-0.9	Survived	Killed 15 days
42 x 7.0	Rat L-511	0.212	34.4	-	Died	During 42nd period of exposure
84 x 7.0	Rat L-512	0.192	27.6	0.0	Survived	Killed 3 days
84 x 7.0	Rat L-513	0.203	26.6	+1.5	Survived	Killed 3 days

Table 4 - Page 3

Duration of Exposure (hours) (1)	Identification Number	Initial Weight (kg)	Change in Weight Expressed as Percentage of Initial Weight		Fate	Length of Survival After Final Period of Exposure Unless otherwise Stated
			During Period of Exposure (1)	After Period of Exposure		
84 x 7.0	Rat L-514	0.195	+ 12.8	0.0	Survived	Killed 3 days
84 x 7.0	Rat L-515	0.217	+ 24.4	0.0	Survived	Killed 3 days
66 x 7.0	Rat L-516	0.210	+ 3.3	-	Survived	Killed 1 day
44 x 7.0	Rat L-748	0.218	+ 22.9	0.0	Survived	Killed 3 days

(1) Computed from time of initial exposure of experimental animals to the vapor of Aroclor; the original group of controls and the original group of experimental animals were confined into conditioned air only, on each of four days during the previous week.

Table 5

Summary of the Data on Mortality Among Groups of Experimental and Control Animals

Material	Concentration mg/l	ppm	Duration of Exposure (hours)	Number of Fatalities/Number of Animals Exposed				
				Cats	Guinea Pigs	Mice	Rabbits	Rats
Aroclor 1242	8.60	0.83	17 x 7.0	0/1	0/6	0/10	0/4	0/10
Aroclor 1242	6.83	0.66	82 x 7.0	0/1	1/7 (1) (2)	3/12 (1) (3)	2/5 (1)	1/11 (1) (4)
Aroclor 1254	5.40	0.41	83 x 7.0	0/1	2/8 (1) (2) (5)	0/10 (1) (6)	3/7 (1) (7)	0/10 (1)
Controls	0	0	84 x 7.0	0/1	2/8 (1) (8)	7/14 (2) (9)	1/5 (10)	1/11 (1) (11)

- (1) One survivor was exposed on only 65 or 66 days.
- (2) One survivor was exposed on only 67 or 68 days.
- (3) Two survivors were exposed on only 41 and 58 days, respectively.
- (4) One survivor was exposed on only 23 days.
- (5) One survivor was exposed on only 71 days.
- (6) Five survivors were exposed on only 74 days.
- (7) One survivor was exposed on only 33 days.
- (8) Two survivors were exposed on only 20 and 77 days, respectively.
- (9) Three survivors were exposed on only 14 and 19 days, respectively.
- (10) One of the survivors was exposed on only 19 days.
- (11) One of the survivors was exposed on only 44 days.

Table 6
The Average Changes in Weight of Experimental and Control Animals

Survivors					Animals That Died					
Species	n	Average Initial Weight (kg)	Average Weight at Termination of Experiment (kg)	Average Change in Weight During Exposure (1) (kg)	Average Change in Weight as Expressed as Percentage of Initial Weight (1)	n	Average Initial Weight (kg)	Average Weight at Termination of Experiment (kg)	Average Change in Weight During Exposure (1) (kg)	Average Change in Weight as Expressed as Percentage of Initial Weight (1)
Group Exposed to 8.6 μ g of Aroclor 1242/liter (Exp. No. 1)										
Cat	1	2.863	3.095	+ 0.232	+ 8.1	0	-	-	-	-
Guinea Pigs	6	0.649	0.640	- 0.008	- 2.0	0	-	-	-	-
Mice	10	0.024	0.026	+ 0.002	+ 9.1	0	-	-	-	-
Rabbits	4	3.901	4.193	+ 0.291	+ 7.7	0	-	-	-	-
Rats	10	0.238	0.243	+ 0.005	+ 2.1	0	-	-	-	-
Group Exposed to 6.03 μ g of Aroclor 1242/liter (Exp. No. 2)										
Cat	1	2.271	2.775	+ 0.504	+ 22.2	0	-	-	-	-
Guinea Pigs	6	0.404	0.629	+ 0.225	+ 56.4	1	0.385	0.321	- 0.064	- 16.6
Mice	9	0.021	0.026	+ 0.004	+ 21.2	3	0.020	0.019	- 0.001	- 7.1
Rabbits	3	2.587	3.398	+ 1.311	+ 50.5	2	2.672	2.968	0.297	+ 11.4
Rats	10	0.212	0.250	+ 0.038	+ 17.6	1	0.213	0.200	- 0.013	- 6.1

Survivors					Animals That Died					
Species	n	Average Initial Weight (kg)	Average Weight at Termination of Experiment (kg)	Average Change in Weight During Exposure (1) (kg)	Average Change in Weight Expressed as Percentage of Initial Weight (1)	n	Average Initial Weight (kg)	Average Weight at Termination of Experiment (kg)	Average Change in Weight During Exposure (1) (kg)	Average Change in Weight Expressed as Percentage of Initial Weight (1)
Group Exposed to 5.4 μ Ci of Aroclor 1254/litter										
Cat	1	2.320	2.833	+ 0.513	+ 22.1	0	-	-	-	-
Guinea Pigs	6	0.409	0.571	+ 0.162	+ 40.7	2	0.402	0.277	- 0.125	- 32.1
Mice	10	0.021	0.027	+ 0.006	+ 28.9	0	-	-	-	-
Rabbits	4	2.722	3.806	+ 1.085	+ 39.7	3	2.569	2.153	- 0.416	- 17.4
Rats	10	0.227	0.242	+ 0.015	+ 6.9	0	-	-	-	-
Group Confined in Conditioned Air Only										
Cat	1	2.388	2.545	+ 0.157	+ 6.6	0	-	-	-	-
Guinea Pigs	6	0.418	0.645	+ 0.227	+ 56.6	2	0.399	0.429	0.030	7.0
Mice	7	0.022	0.024	+ 0.002	+ 17.4	6	0.022	0.020	- 0.001	- 6.1
Rabbits	4	2.764	4.012	+ 1.248	+ 44.5	1	3.352	3.822	0.470	14.0
Rats	10	0.211	0.246	+ 0.034	+ 16.1	1	0.212	0.139	- 0.073	- 34.4

$$(1) \left(\frac{\sum (\text{Final Weight} - \text{Initial Weight} \times 100\%)}{n} \right)$$

Table 7

The Relationships of the Weights of Certain Organs to the Body Weights of Animals Exposed to the Vapor of Aroclor 1242 or Aroclor 1254

Identification Number	Number of Periods of Exposure	Body Weight (g)	Liver		Kidneys	
			g	Ratio of Weight of Organ x 100 to Body Weight	g	Ratio of Weight of Organ x 100 to Body Weight
Aroclor 1242 6.83 Micrograms/liter						
Cat A-445	82	2775	92.0	3.30	17.5	0.631
Guinea Pig B-325	82	639	23.9	3.74	3.4	0.532
Guinea Pig B-327	82	798	31.4	3.93	4.9	0.614
Guinea Pig B-320	82	609	31.0	5.09	6.0	0.985
Guinea Pig B-329	66	412	22.0	5.34	4.5	1.092
Guinea Pig B-330	82	698	35.0	5.01	6.2	0.888
Guinea Pig B-419	67	683	34.0	4.98	4.9	0.717
Average	77	610	29.6	4.68	5.0	0.805
Rabbit I-370	82	4265	112.0	2.63	14.5	0.340
Rabbit I-371	82	4300	114.0	3.35	21.5	0.500
Rabbit I-373	66	3505	110.0	3.14	19.8	0.565
Average	77	4023	122.0	3.04	18.6	0.468

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Identification Number	Number of Periods of Exposure	Body Weight (g)	Liver		Kidneys	
			g	Ratio of Weight of Organ x 100 to Body Weight	g	Ratio of Weight of Organ x 100 to Body Weight

Aroclor 1242 6.83 Micrograms/liter						
Rat L-526	65	240	12.5	5.21	2.01	0.833
Rat L-527	82	253	11.4	4.51	1.75	0.692
Rat L-528	82	254	11.0	4.33	1.81	0.709
Rat L-529	82	202	8.7	4.31	1.85	0.916
Rat L-531	82	220	10.0	4.55	1.60	0.727
Rat L-533	82	269	10.5	3.90	1.81	0.669
Rat L-534	82	277	14.0	5.05	2.20	0.794
Rat L-535	82	228	14.9	6.54	2.30	1.009
Rat L-836	23	221	7.5	3.39	1.34	0.606
Average	74	240	11.2	4.64	1.85	0.773

Aroclor 1254 5.40 Micrograms/liter

Cat A-444	83	2833	93.3	3.29	16.0	0.565
Guinea Pig B-319	83	566	22.9	4.05	4.6	0.813
Guinea Pig B-320	83	640	27.3	4.27	5.2	0.813
Guinea Pig B-321	83	682	26.3	3.86	4.2	0.616
Guinea Pig B-324	66	510	26.0	5.10	4.8	0.941
Guinea Pig B-415	71	462	23.2	5.02	4.0	0.866

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Identification Number	Number of Periods of Exposure	Body Weight (g)	Liver		Kidneys	
			g	Ratio of Weight of Organ x 100 to Body Weight	g	Ratio of Weight of Organ x 100 to Body Weight

Aroclor 1254 5.40 Micrograms/liter

Guinea Pig						
B-418	69	628	29.7	4.73	5.0	0.796
Average	76	591	25.9	4.51	4.6	0.808
Rabbit I-362	66	2975	184.0	6.18	24.0	0.607
Rabbit I-363	83	4116	118.0	2.84	15.1	0.367
Rabbit I-364	83	4185	103.0	2.46	14.5	0.346
Rabbit I-887	33	2870	75.1	2.62	9.6	0.334
Average	66	3537	120.0	3.53	15.8	0.464
Rat I-299	83	241	13.0	5.39	2.0	0.830
Rat I-517	66	220	11.4	5.18	1.4	0.636
Rat I-518	83	214	10.7	5.00	1.6	0.748
Rat I-519	83	278	16.6	5.97	2.1	0.755
Rat I-520	83	242	17.2	7.11	2.2	0.909
Rat I-521	83	228	12.2	5.35	3.5	1.535
Rat I-522	83	255	10.6	4.16	1.9	0.745
Rat I-523	83	232	11.4	4.91	1.8	0.776
Rat I-524	83	242	13.1	5.41	2.2	0.909
Rat I-525	83	233	11.4	4.89	2.0	0.858
Average	81	239	12.8	5.34	2.1	0.770

Controls

Cat A-435	84	2545	48.5	1.91	14.4	0.566

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Identification Number	Number of Periods of Exposure	Body Weight (g)	Liver		Kidneys	
			g	Ratio of Weight of Organ x 100 to Body Weight	g	Ratio of Weight of Organ x 100 to Body Weight
Controls						
Guinea Pig						
B-313	84	654	25.1	3.84	4.6	0.703
Guinea Pig						
B-314	66	519	18.2	3.51	4.6	0.886
Guinea Pig						
B-315	84	738	36.4	4.93	4.0	0.542
Guinea Pig						
B-317	84	870	40.8	4.69	5.7	0.655
Guinea Pig						
B-402	77	626	29.7	4.74	4.9	0.783
Guinea Pig						
B-576	20	588	31.3	5.32	4.8	0.816
Average	69	666	30.3	4.51	4.8	0.731
Rabbit I-365	84	4505	124.0	2.75	18.8	0.417
Rabbit I-366	84	5000	104.0	2.08	20.3	0.406
Rabbit I-368	84	3490	95.5	2.74	21.8	0.625
Rabbit I-975	19	2973	85.7	2.88	16.9	0.568
Average	68	3993	102.3	2.61	19.5	0.504
Rat L-507	84	203	8.2	1.04	1.7	0.837
Rat L-508	84	245	8.4	3.43	1.7	0.694
Rat L-509	84	241	9.5	3.94	2.3	0.954
Rat L-510	84	266	10.5	3.95	1.9	0.714

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Identification Number	Number of Periods of Exposure	Body Weight (g)	Liver		Kidneys		
			g	Ratio of Weight of Organ x 100 to Body Weight	g	Ratio of Weight of Organ x 100 to Body Weight	
Controls							
Rat L-512	84	245	8.1	3.31	2.2	0.898	
Rat L-513	84	257	10.2	3.97	2.4	0.934	
Rat L-514	84	220	12.9	5.86	2.1	0.954	
Rat L-515	84	270	9.9	3.67	1.9	0.704	
Rat L-516	66	217	9.2	4.24	1.3	0.599	
Rat L-748	40	268	13.5	5.04	2.1	0.784	
Average	78	243	9.0	4.15	2.0	0.807	

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Table 8

Summary of the Data on the Relationship of the Weight of the Liver or the Kidneys to the Body Weight of Animals Exposed to the Vapor of Aroclor 1242 or Aroclor 1254

Aroclor	Organ	Species	n	Average Organ Weight (g)	Average Body Weight (g)	Ratio of Weight of Organ x 100 to Body Weight	t	P
1242	Liver	Rat	9	11.2	240	4.64	1.2381	0.20-0.30
1242	Liver	Guinea Pig	6	29.6	640	4.68	0.4291	>0.50
1242	Liver	Rabbit	3	122.0	4023	3.04	1.5085	0.10-0.20
1254	Liver	Rat	10	12.8	239	5.34	3.3992	>0.01
1254	Liver	Guinea Pig	6	25.9	581	4.51	0.0000	>0.50
1254	Liver	Rabbit	4	120.0	3537	3.53	1.0122	>0.50
1242	Kidney	Rat	9	1.85	240	0.773	0.5842	>0.50
1242	Kidney	Guinea Pig	6	5.0	640	0.805	0.7257	0.40-0.50
1242	Kidney	Rabbit	3	18.6	4023	0.468	0.4177	>0.50
1254	Kidney	Rat	10	2.1	239	0.770	0.4199	>0.50
1254	Kidney	Guinea Pig	6	4.6	581	0.808	0.5411	>0.50
1254	Kidney	Rabbit	4	15.8	3537	0.464	0.3163	>0.50
1254	Liver	Rat	10	9.0	243	4.15	-	-
1254	Liver	Guinea Pig	6	30.3	266	4.51	-	-
1254	Liver	Rabbit	4	102.3	3993	2.61	-	-
1254	Kidney	Rat	10	2.0	243	0.807	-	-
1254	Kidney	Guinea Pig	6	4.8	666	0.731	-	-
1254	Kidney	Rabbit	4	19.5	3993	0.504	-	-

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{n_1 n_2 (n_1 + n_2 - 2)}{(n_1 + n_2) (Sx_1^2 + Sx_2^2)}}} ; df = n_1 + n_2 - 2$$

NEV 016596

Table 9

The "Prothrombin Time" (Method of Kato) of the
Blood of Animals Following Their Exposure to the
Vapor of Aroclor 1242 or Aroclor 1254 in Air

NEV 016597

Date	Clotting Time, Seconds				
	Experiment No. 2 Aroclor 1242 - 6.83 γ /l				
	Cat A-445	Rabbits			
		I-370	I-371	I-372	I-373
9/11/53	20.00	19.00	19.50	19.50	18.50
9/12/53	18.50	17.00	17.00	18.00	16.50
9/26/53	18.00	13.50	15.00	13.50	13.50
10/24/53	16.50	15.00	15.00	14.50	14.00
11/24/53	17.00	14.00	13.50	13.50	14.00
12/5/53	19.00	16.50	15.00	16.00	16.75
1/12/54	17.00	16.00	17.00		16.75
1/26/54			15.00		

Table 9 - Page 2

NEV 016598

Date	Clotting Time, Seconds							
	Experiment No. 1 Aroclor 1254 - 5.40 7/1							
	Rabbits							
	Cat A-4444	I-362	I-363	I-364	I-453	I-587	I-718	I-887
9/11/53	20.00	17.00	18.00	17.00	17.00			
9/12/53	20.00	16.00	16.50	18.00	17.00			
9/26/53	16.50	16.00	16.00	15.00	18.25			
10/24/53	16.50	15.00	14.00	15.00		24.00		
11/14/53	17.00	15.00	14.00	13.50			14.00	16.50
12/5/53	20.00	17.25	16.50	16.50				
12/16/53								
1/13/54								
1/26/54	20.00		15.00	15.00				15.00

Table 10
The "Prothrombin Time" (Method of Keto) of the Blood of Animals Following Their
Confinement in Cages Supplied with Conditioned Air Containing No Vapor of
Aroclor 1242 or Aroclor 1254

Date	Cat A-435	Clotting Time, Seconds				
		Rabbits				
		I-365	I-366	I-368	I-369	I-975
						Average
9/11/53	20.00	16.00	16.00	16.00	18.50	16.63
9/12/53	20.00	16.00	16.50	16.00	16.25	16.19
9/26/53	16.25	13.25	13.50	15.50	14.75	14.25
10/24/53	18.75	13.50	14.50	14.50	15.00	14.38
11/14/53	17.25	14.50	13.00	13.50	13.00	13.50
12/5/53	19.50	15.50	16.25	15.75	17.00	16.13
1/12/54	19.75	16.75	15.50	17.75		16.81
1/13/54	18.00	15.50	16.00	14.25		14.94
1/26/54				15.00		15.00

665910 016599

Table 11
The Effect of the Exposure of Cats and Rabbits to the Vapor of Aroclor 1242
or Aroclor 1254 in Air for 7 Hours per Day on 5 Days per Week Over a Per-
iod of Several Months, Upon the Apparent Prothrombin Content (Method of Katz) of their Blood

NEV 016600

Relation of "Prothrombin Time" of Control and Exposed Animals Expressed Arbitrarily in Terms of Percentage Change in Clotting Time of Blood

100 x Clotting Time of Blood of Control Animals
 Clotting Time of Blood of Exposed Animals

Time of Sampling in Relation to Period of Exposure	Days Before First Period	Serial Number of Period Which Blood Was Drawn

Experiment No. 2 Aroclor 1242 - 6.83 7/1

Cat A-445	Rabbits				Average		
	I-370	I-371	I-372	I-373			
100.0	87.5	85.3	85.3	89.9	89.6	2	10
90.3	95.2	95.2	89.9	90.1	93.8		
113.6	105.6	95.0	105.6	105.6	105.1		
101.5	95.9	95.9	99.2	102.7	99.0		
97.4	96.4	100.0	100.0	96.4	98.0		
116.2	97.8	107.5	100.8	96.3	103.7		45
	105.1	98.9		100.4	101.5		52
		100.0			100.0		82
							(1)

(1) Determination made 15 days after 82nd period of exposure.

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NEV 016601

Time of Sampling in Relation to Period of Exposure		Time of Clotting Time of Blood of Exposed Animals	
Days Before First Period	Serial Number of Period After Which Blood Was Drawn	100 x Clotting Time of Blood of Control Animals	Clotting Time of Blood of Exposed Animals

Experiment No. 1 Arcolor 1254 - 5.40 7/1

Cat		Rabbits						Average	3	9 15 30 45 59 83 (2)
A-4444	I-362	I-363	I-364	I-453	I-587	I-718	I-887			
100.0	97.8	92.3	97.8	97.8				97.1		
100.0	101.2	98.1	89.9	95.2				81.8		
98.5	89.1	89.1	95.0	78.1				90.0		
113.6	95.9	102.7	95.9		102.7	96.4		99.6		
								102.0		
101.5	90.0	96.4	100.0					112.1		
92.5	93.5	97.8	97.8					97.0		
90.0		100.4	100.0					95.4		
								100.0		

(2) Determination made 14 days after 83rd period of exposure.

Table 12

The Effects of the Intermittent Inhalation of the Vapor of Aroclor 1242 or Aroclor 1254, or of Conditioned Air, Upon the Numbers of Erythrocytes and Leucocytes and Upon the Concentration of Hemoglobin in the Peripheral Blood of Animals

Experiment No. 2 Aroclor 1242 - 6.83%/1: 7 hours/day

Identification Number	Dates of First and Last Period of Exposure	Date of Determination	Hemoglobin g/100 ml	Erythrocytes (Thousands) per mm ³	Leucocytes per mm ³
Cat A-445	9/14/53	9/17/53	12.4	8,050	20,950
		10/28/53	10.3	7,180	12,700
		12/22/53	10.0	7,180	15,050
		Average	10.9	7,470	16,218
Guinea Pig B-325	9/14/53	9/18/53	13.8	5,480	16,350
		10/29/53	15.4	6,540	10,300
		12/21/53	14.2	6,040	11,200
		Average	14.5	6,010	12,617
Guinea Pig B-326	9/14/53	9/16/53	15.8	5,830	16,750
		10/2/53			
Guinea Pig B-327	9/14/53	9/16/53	14.6	5,260	6,400
		10/29/53	15.8	6,620	11,300
		12/21/53	15.8	6,030	8,200
		Average	15.4	5,970	8,633

016602

Table 12 - Page 2

Experiment No. 2 Aroclor 1242 - 6,837/1: 7 hours/day

Identification Number	Dates of First and Last Period of Exposure	Date of Determination	Hemoglobin g/100 ml	Erythrocytes (Thousands) per mm ³	Leucocytes per mm ³
Guinea Pig B-328	9/14/53	9/16/53 10/29/53 12/5/53	12.7 13.1 13.4	5,290 5,550 5,620	10,300 8,150 13,850
	1/11/54	Average	13.1	5,487	10,767
Guinea Pig B-329	9/14/53	9/16/53 10/29/53 12/12/53	15.0 13.1 14.2	5,150 5,500 5,420	5,700 9,150 11,950
	12/15/53	Average	14.1	5,357	5,600
Guinea Pig B-330	9/14/53	9/18/53 10/29/53 12/21/53	12.0 15.4 13.8	4,930 6,370 5,740	6,500 8,300 10,000
	1/11/54	Average	13.7	5,680	8,267
Guinea Pig B-419	10/5/53	12/21/53	14.2	5,570	8,150
	1/11/54				
Rabbit I-370	9/14/53	9/16/53 10/28/53 12/22/53	11.6 13.4 12.7	5,700 6,900 5,590	9,400 15,400 10,600
	1/11/54	Average	12.6	6,090	11,800

NEV 016603

Experiment No. 2 Arcolior 1242 - 6.83 V/l: 7 hours/day

Identifica- tion Number	Dates of First and Last Period of Exposure	Date of Determi- nation	Hemoglobin g/100 ml	Erythrocytes (Thousands) per mm ³	Leucocytes per mm ³
Rabbit I-371	9/14/53 1/11/54	9/16/53 10/28/53 12/22/53 Average	12.0 11.4 12.4 11.9	5,900 5,940 6,030 5,957	12,250 12,300 7,350 10,633
Rabbit I-372	9/14/53 12/11/53	9/16/53 10/28/53 Average	11.6 13.1 12.4	5,970 7,090 6,530	8,950 14,000 11,475
Rabbit I-373	9/14/53 12/15/53	9/16/53 10/28/53 12/9/53 Average	11.6 11.6 13.1 12.1	5,540 6,040 5,140 5,573	8,150 10,450 8,600 9,067
Rabbit I-964	12/15/53 1/7/54	12/22/53	11.6	6,290	5,800
Experiment No. 1 Arcolior 1294 - 5.40 V/l: 7 hours/day					
Cat A-444	9/14/53	9/18/53	11.6	9,540	25,900

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NEV 016605

Experiment No. 1 Aroclor 1254 - 5.407/1: 7 hours/day

Identification Number	Dates of First and Last Period of Exposure	Date of Determination	Hemoglobin g/100 ml.	Erythrocytes (Thousands) per mm ³	Leucocytes per mm ³
Cat A-444	1/12/54	10/30/53 12/23/53 Average	12.0 13.4 12.3	8,390 7,550 8,493	25,100 26,350 25,783
Guinea Pig B-319	9/14/53	9/21/53 11/2/53 12/22/53 Average	15.0 15.0 13.4 14.5	5,780 5,970 5,580 5,777	16,000 9,550 11,350 12,300
Guinea Pig B-320	9/14/53	9/21/53 11/2/53 12/22/53 Average	14.6 15.8 13.8 14.7	5,760 5,690 5,310 5,587	12,100 8,550 16,550 12,400
Guinea Pig B-321	9/14/53	9/21/53 11/2/53 12/22/53 Average	14.6 14.6 15.4 14.9	5,450 6,190 6,170 5,937	6,350 16,650 12,100 11,700
Guinea Pig B-322	9/14/53	9/21/53	13.4	5,760	14,950
Guinea Pig B-323	9/29/53 9/29/53	9/21/53	15.0	6,270	8,700

Table 12 - Page 5

Experiment No. 1 Aroclor 1254 - 5.40 γ/l: 7 hours/day

Identification Number	Dates of First and Last Period of Exposure	Date of Determination	Hemoglobin g/100 ml	Erythrocytes (Thousands) per mm ³	Leucocytes per mm ³
Guinea Pig B-324	9/14/53	9/21/53 11/2/53 12/12/53	15.0 13.8 14.2	5,880 6,200 6,110	14,500 16,800 15,200
	12/15/53	Average	14.3	6,063	15,167
Guinea Pig B-415	9/30/53	11/2/53 12/22/53	14.2 14.6	5,930 5,710	22,900 12,900
	1/12/54	Average	14.4	5,820	17,900
Guinea Pig B-418	10/5/53	11/2/53 12/22/53	14.6 14.2	6,330 5,410	10,000 10,600
	1/12/54	Average	14.4	5,870	10,300
Rabbit I-362	9/14/53	9/16/53 10/30/53 12/9/53	14.2 13.4 13.1	6,850 6,020 5,960	15,300 12,400 13,100
	12/15/53	Average	13.6	6,277	13,600
Rabbit I-363	9/14/53	9/18/53 10/30/53 12/23/53	13.4 11.6 11.6	6,770 5,960 5,470	7,400 10,450 12,500
	1/12/54	Average	12.2	6,067	10,117

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Experiment No. 1 Aroclor 1254 - 5.407/1: 7 hours/day

Identification Number	Dates of First and Last Period of Exposure	Date of Determination	Hemoglobin g/100 ml	Erythrocytes (Thousands) per mm ³	Leucocytes per mm ³
Rabbit I-364	9/14/53	9/18/53 10/30/53 12/23/53	13.4 12.4 12.4	6,430 6,890 6,300	6,050 13,800 10,000
	1/12/54	Average	12.7	6,540	9,950
Rabbit I-453	9/14/53	9/18/53	15.0	6,900	11,050
	10/2/53				
Rabbit I-587	10/5/53 10/23/53				
Rabbit I-718	10/26/53	10/30/53	11.6	6,500	10,850
	11/20/53 11/23/53				
Rabbit I-887	1/12/54	12/23/53	13.8	6,630	9,300

Conditioned Air

Cat A-435	9/8/53	9/22/53 11/3/53 12/23/53	10.0 10.6 11.0	7,970 7,140 7,200	25,050 14,450 12,350
	1/13/54	Average	10.5	7,437	17,283

NEV 016607

Table 12 - Page 7

Conditioned Air

Identification Number	Dates of First and Last Period of Exposure	Date of Determination	Hemoglobin g/100 ml	Erythrocytes (Thousands) per mm ³	Leucocytes per mm ³
Guinea Pig B-313	9/8/53	9/23/53 11/4/53 12/23/53	13.4 13.8 15.4	5,490 6,080 5,870	11,250 15,050 11,150
	1/13/54	Average	14.2	5,813	12,483
Guinea Pig B-314	9/8/53	9/23/53 11/4/53 12/12/53	12.0 14.2 ---	5,290 5,780 5,230	12,150 14,800 19,250
	12/15/54	Average	13.1	5,433	15,400
Guinea Pig B-315	9/8/53	9/23/53 11/4/53 12/23/53	13.8 14.6 14.2	5,790 5,510 5,540	14,950 17,050 17,050
	1/13/54	Average	14.2	5,613	15,867
Guinea Pig B-316	9/8/53 9/22/53				
Guinea Pig B-317	9/8/53	9/23/53 11/4/53 12/23/53	13.4 15.0 15.0	5,970 6,960 6,590	6,000 5,450 10,450
	1/13/54	Average	14.5	6,513	7,300

Table 12 - Page 8

Conditioned Air

Identification Number	Dates of First and Last Period of Exposure	Date of Determination	Hemoglobin g/100 ml.	Erythrocytes (Thousands) per mm ³	Leucocytes per mm ³
Guinea Pig B-318	9/8/53	9/23/53 11/4/53	13.4 12.7	5,520 5,320	9,900 17,000
	12/4/53	Average	13.1	5,420	13,450
Guinea Pig B-402	9/23/53	9/23/53 11/4/53 12/23/53	13.1 13.8 15.4	5,320 5,210 5,830	10,100 10,650 12,150
	1/13/54	Average	14.1	5,453	10,967
Guinea Pig B-576	12/14/53	12/23/53	14.2	5,860	9,400
	1/13/54	9/22/53 11/3/53 12/23/53	11.4 12.4 13.4	5,900 5,800 6,200	8,900 8,100 11,450
Rabbit I-365	9/8/53	Average	12.4	5,967	9,483
	1/13/54	9/22/53 11/3/53 12/23/53	11.4 11.4 12.7	5,860 5,760 5,280	16,400 16,250 13,750
Rabbit I-366	9/8/53	Average	11.6	5,633	15,467
	1/13/54	Average			

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Table 12. - Page 9

Conditioned Air

Identification Number	Dates of First and Last Period of Exposure	Date of Determination	Hemoglobin g/100 ml	Erythrocytes (Thousands) per mm ³	Leucocytes per mm ³
Rabbit I-368	9/8/53	9/22/53 11/3/53 12/23/53	11.4 11.4 12.4	5,630 5,060 6,240	9,100 10,900 13,100
	1/13/54	Average	11.7	5,643	11,033
Rabbit I-369	9/8/53	9/22/53 11/3/53 12/9/53	12.4 12.0 11.6	6,490 5,900 5,260	11,700 9,100 16,300
	12/14/54	Average	12.0	5,883	12,367
Rabbit I-975	12/15/53 1/13/54	12/23/53	13.1	6,420	9,600

Table 13

The Average Numbers of Erythrocytes and Leucocytes and the Average Concentration of Hemoglobin in the Peripheral Blood of Experimental and Control Animals

NEV 016611

Species of Animals	Erythrocytes: Thousands per mm ³		Leucocytes: per mm ³		Hemoglobin: g/100 ml	
	Average $\bar{x}$	n	Average $\bar{x}$	n	Average $\bar{x}$	n
Experiment No. 2 Aroclor 1242 - 6.85 ✓/1. : 7 hours/day						
Guinea Pigs	5,701	7	10,112 (1)	7	14.4 (2)	7
Rabbits	6,088	5	9,755	5	12.1	5
Experiment No. 1 Aroclor 1254 - 5.40 ✓/1. : 7 hours/day						
Guinea Pigs	5,886	8	12,927	8	14.5 (2)	8
Rabbits	6,486 (2)	6	10,811	6	13.2	6
Controls Exposed to Conditioned Air						
Guinea Pigs	5,729	7	12,124	7	13.9	7
Rabbits	5,909	5	11,590	5	12.2	5

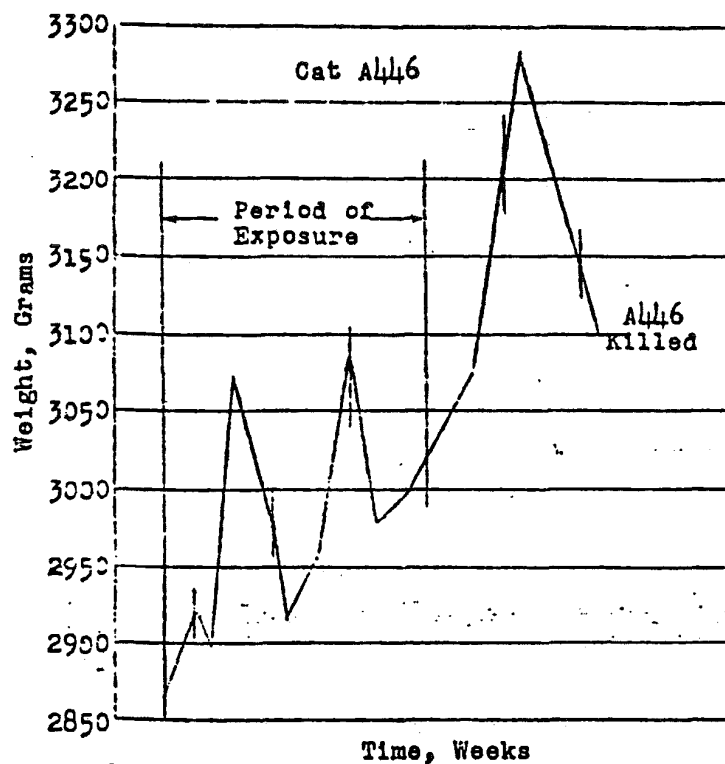
- (1) Value significantly less than that obtained on controls.
 (2) Value significantly greater than that obtained on controls.

Application of the "t" Test to the Hematological Data
Obtained in Observations on Exposed and Control Animals

Table 14

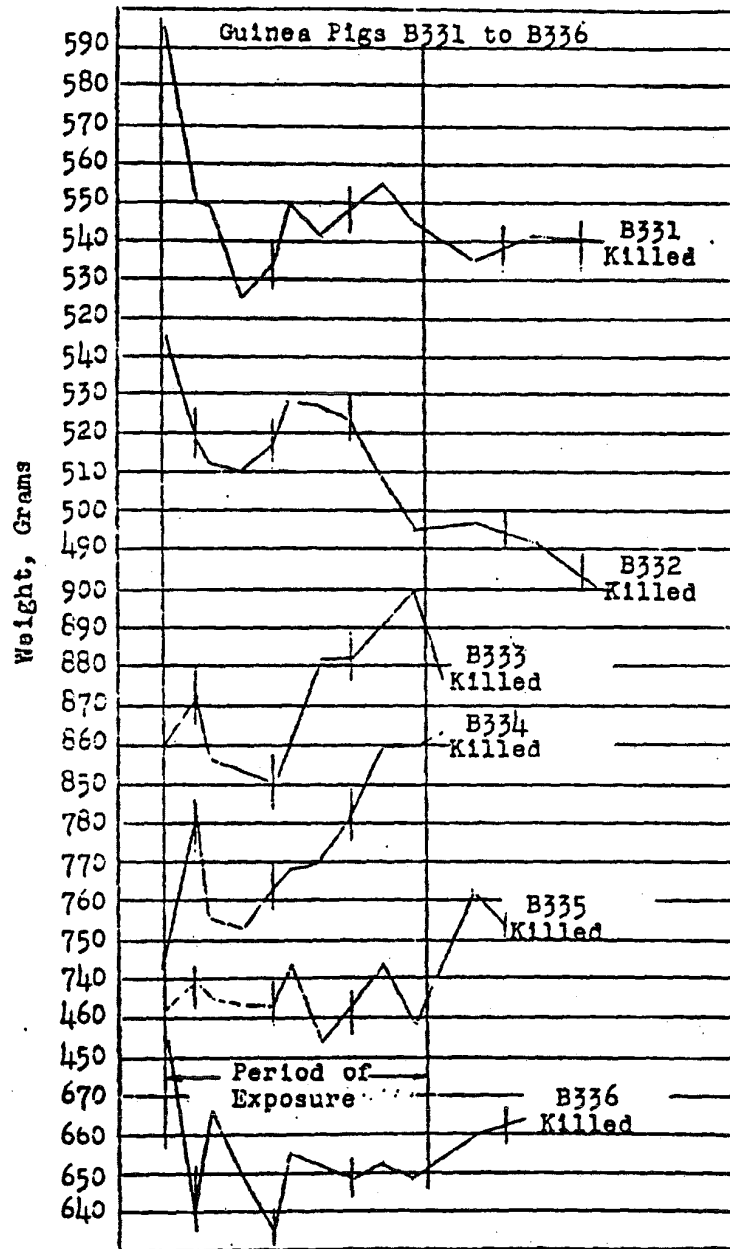
Species of Animals	Erythrocytes thousands/mm ³			Leucocytes Per mm ³			Hemoglobin g/100 ml		
	df	t	P	df	t	P	df	t	P
Experiment No. 2 Aroclor 1242 - 6.83 v/1: 7 hours/day									
Guinea Pigs	12	0.1604	>0.50	12	2.7894	0.01-0.02	12	3.5496	<0.01
Rabbits	8	0.8298	0.40-0.50	8	1.4371	0.10-0.20	8	0.3086	>0.50
Experiment No. 1 Aroclor 1254 - 5.40 v/1: 7 hours/day									
Guinea Pigs	13	0.9924	0.30-0.40	13	0.5117	>0.50	13	2.1731	0.02-0.05
Rabbits	9	3.1443	0.01-0.02	9	0.6457	>0.50	9	1.6423	0.20-0.10

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{n_1 n_2 (n_1 + n_2 - 2)}{(n_1 + n_2) (Sx_1^2 + Sx_2^2)}}}; \quad df = n_1 + n_2 - 2$$



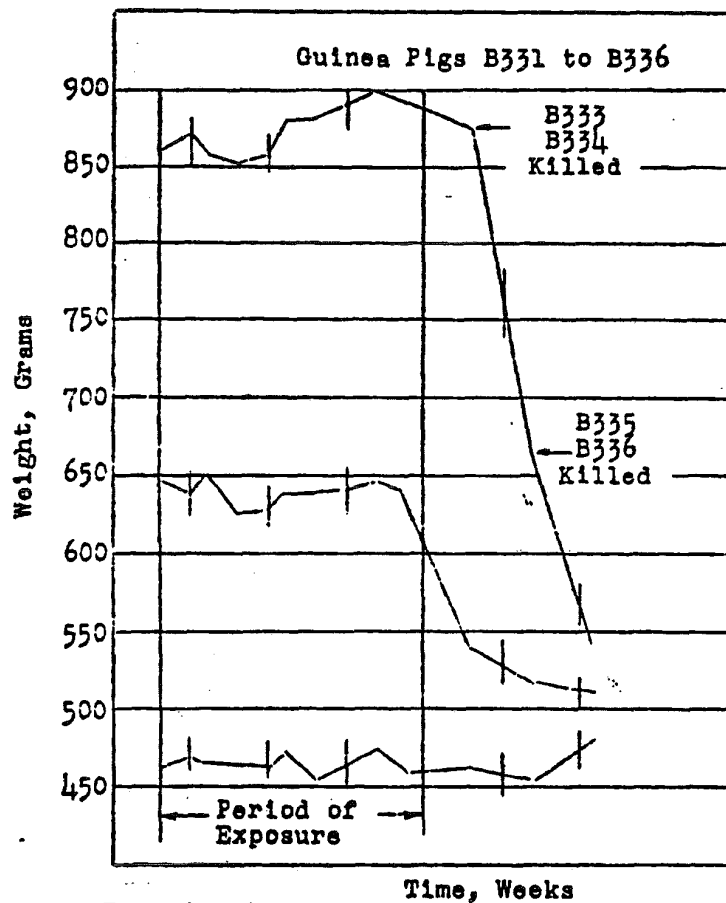
Experiment No. 1
8.6 Micrograms of Aroclor 1242/liter
Figure 1

NEV 016613



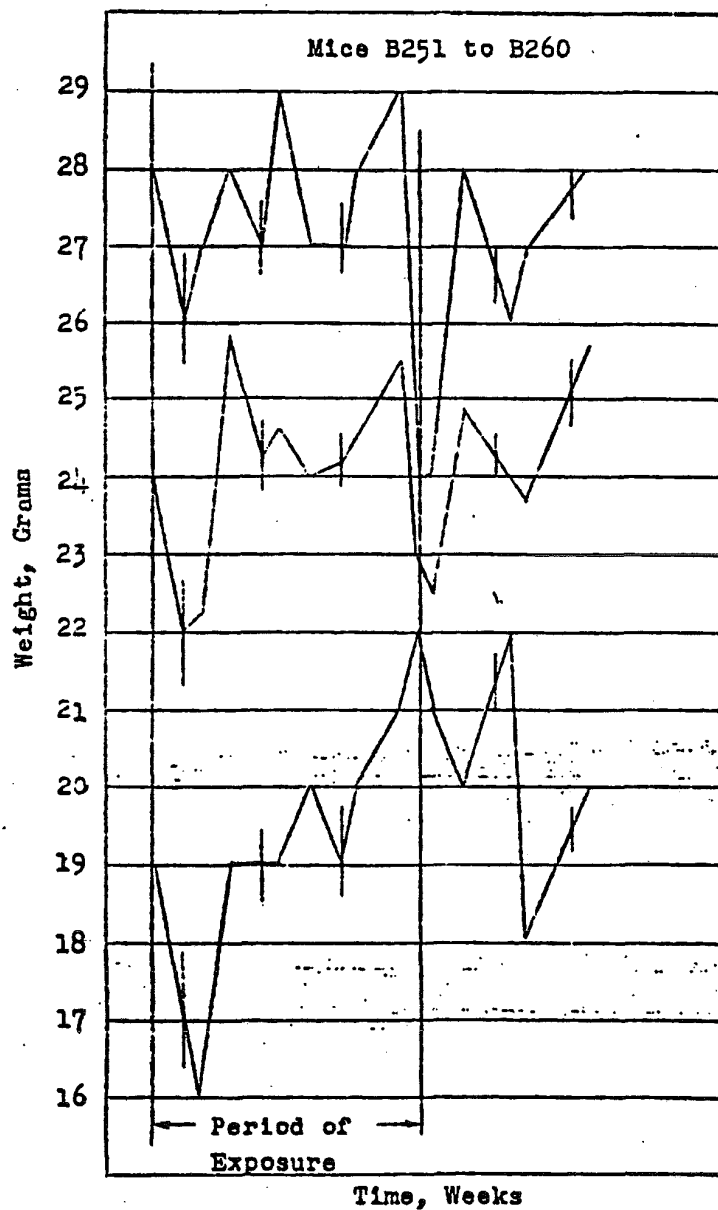
Time, Weeks
 Experiment No. 1
 8.6 Micrograms of Aroclor 1242/liter
 Figure 2

NEV 016614



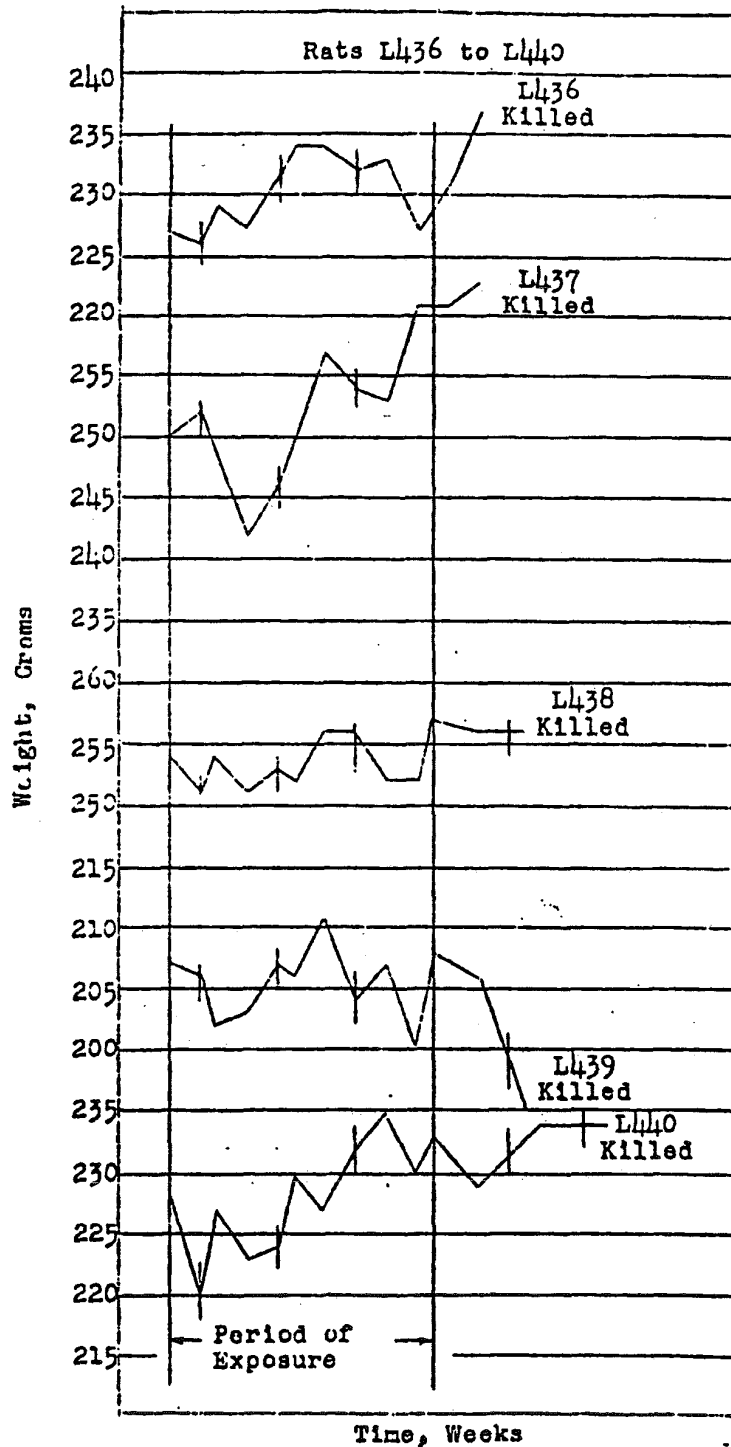
Experiment No. 1
 8.6 Micrograms of Aroclor 1242/liter
 Figure 3

NEV 016615



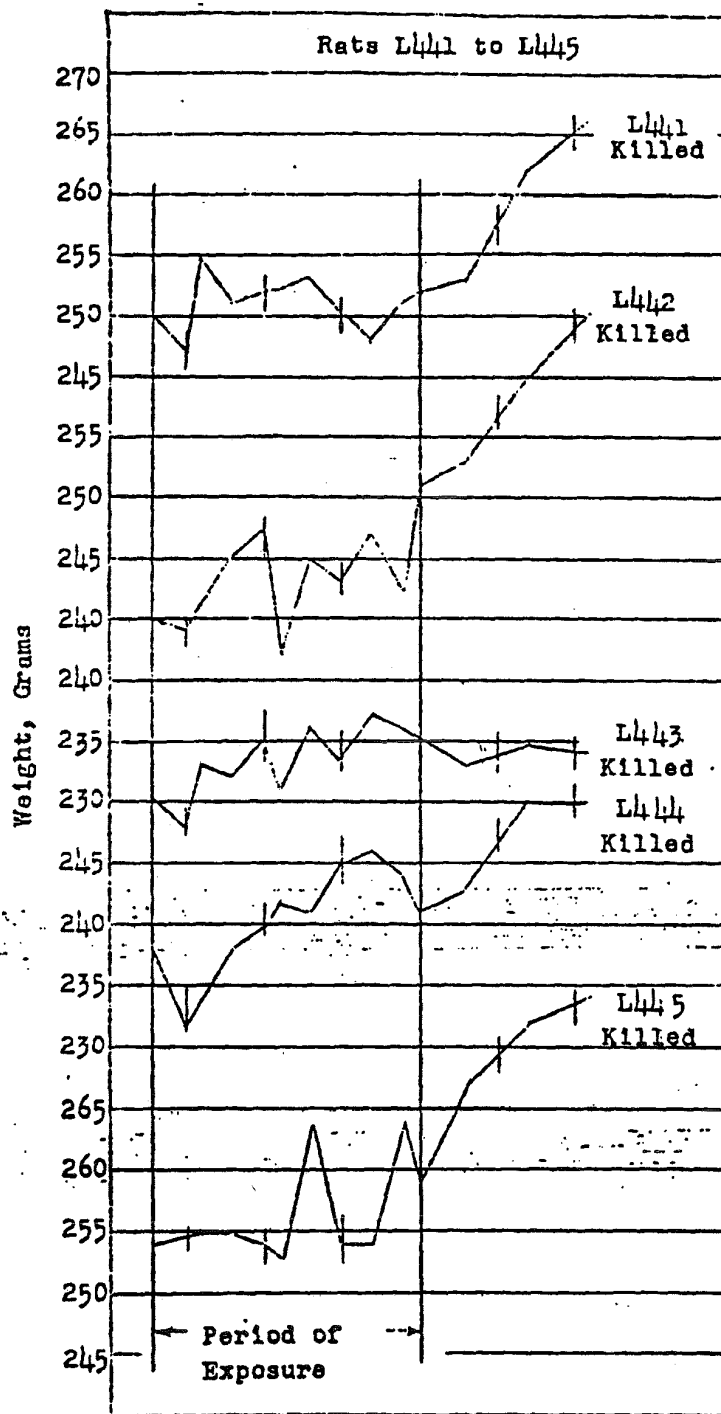
Experiment No. 1
8.6 Micrograms of Aroclor 1242/liter
Figure 4

NEV 016616



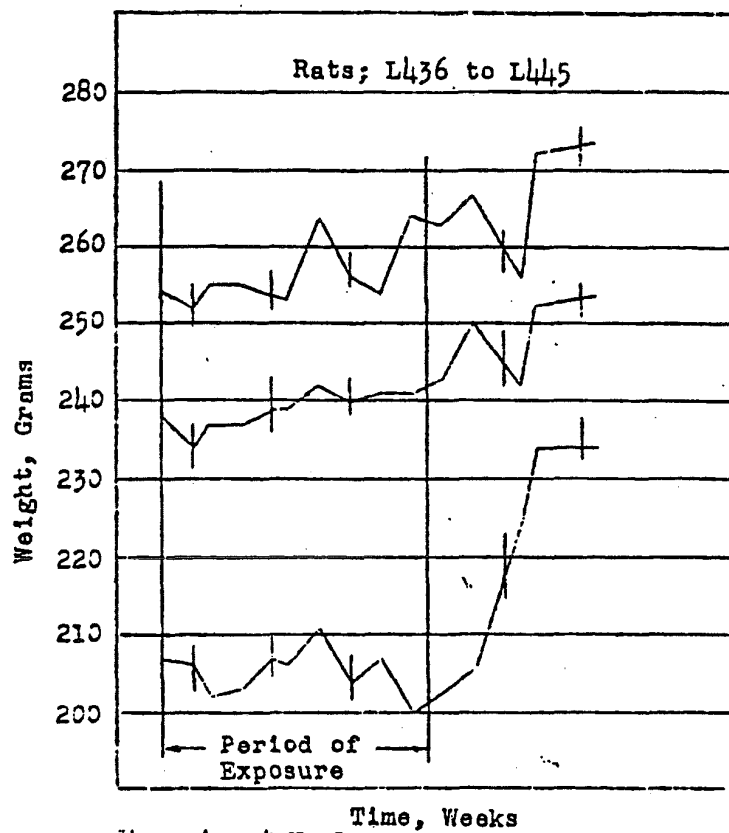
Experiment No. 1
 8.6 Micrograms of Aroclor 1242/liter
 Figure 5

NEV 016617



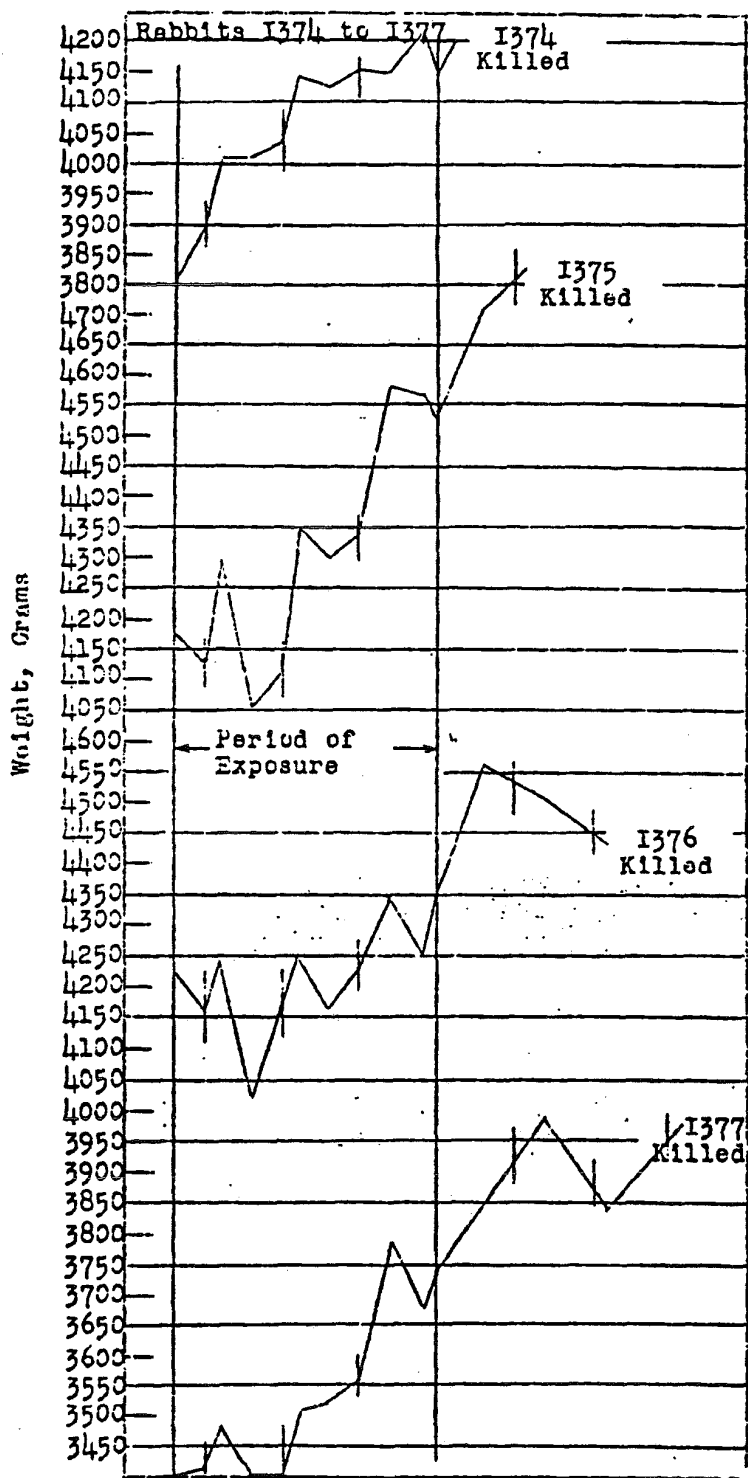
Experiment No. 1
 8.6 Micrograms of Aroclor 1242/liter
 Figure 6

NEV 016618



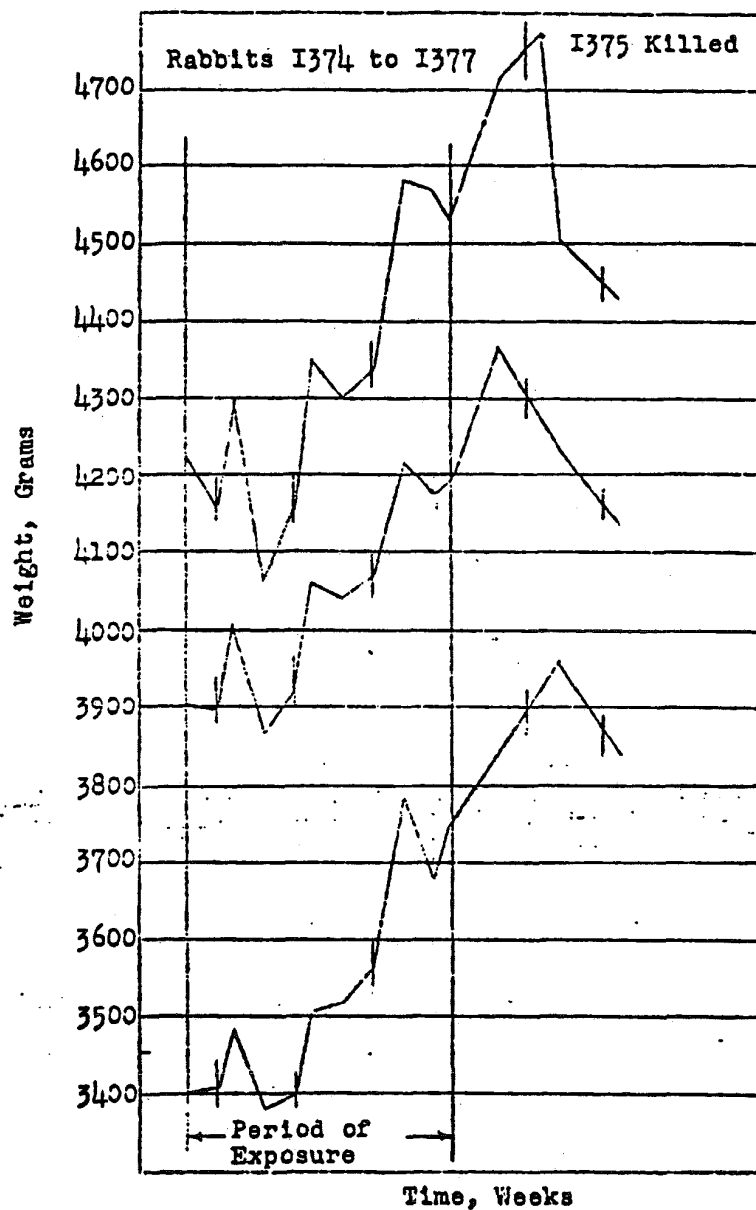
Experiment No.1
8.6 Micrograms of Aroclor 1242/liter
Figure 7

NEV 016619



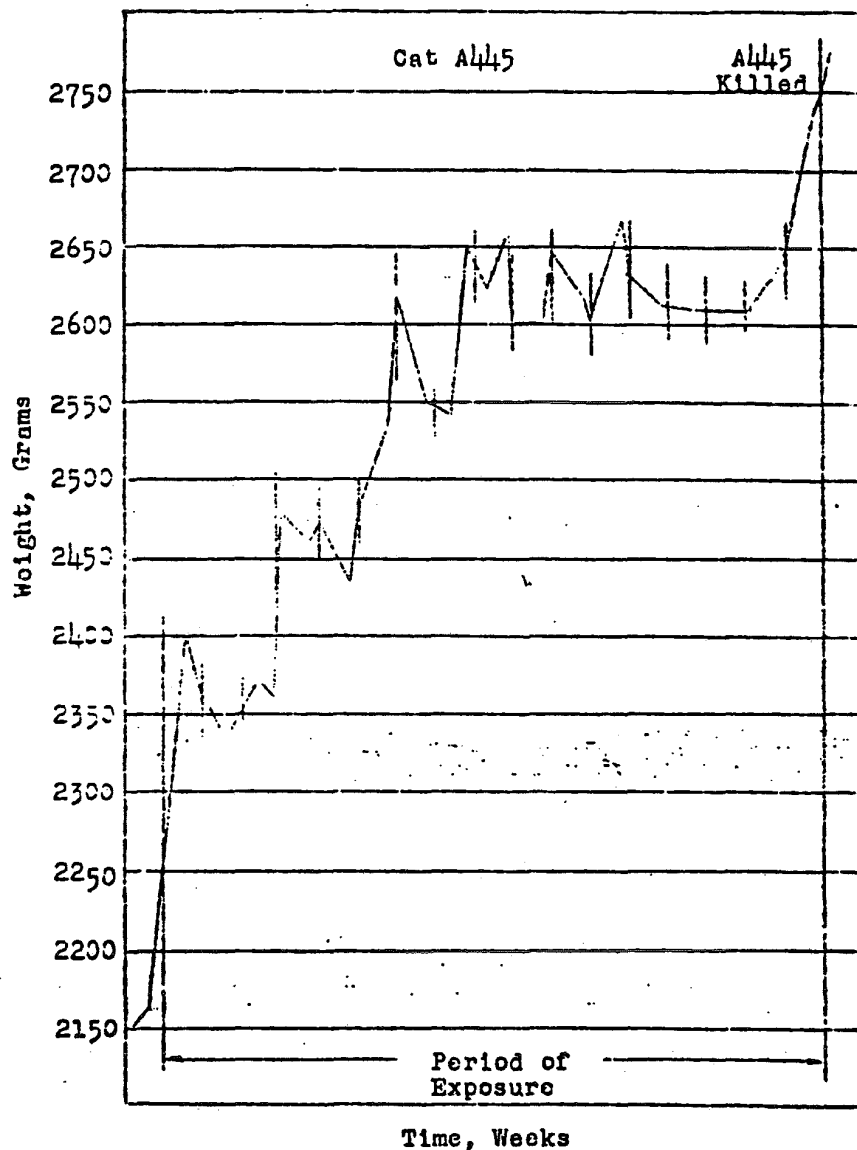
Experiment No. 1
8.6 Micrograms of Aroclor 1242/liter

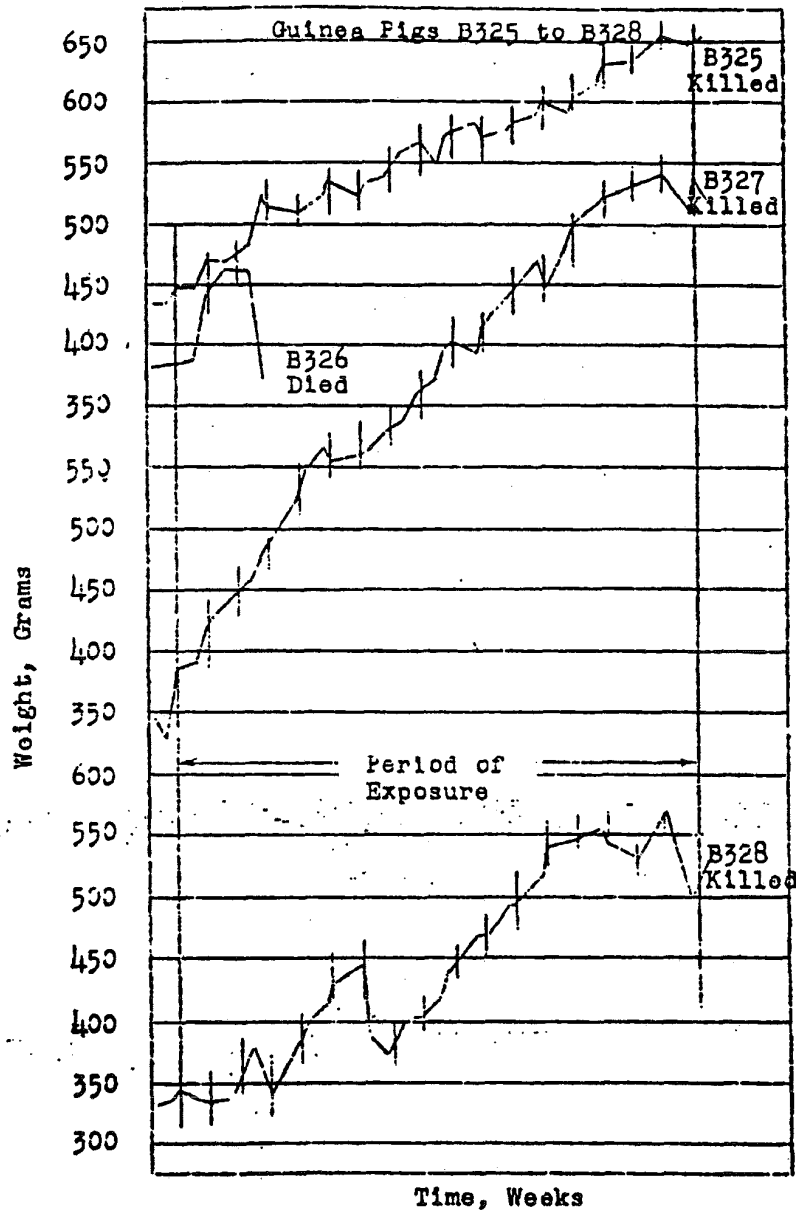
NEW 016620



Experiment No. 1
8.6 Micrograms of Aroclor 1242/liter
Figure 9

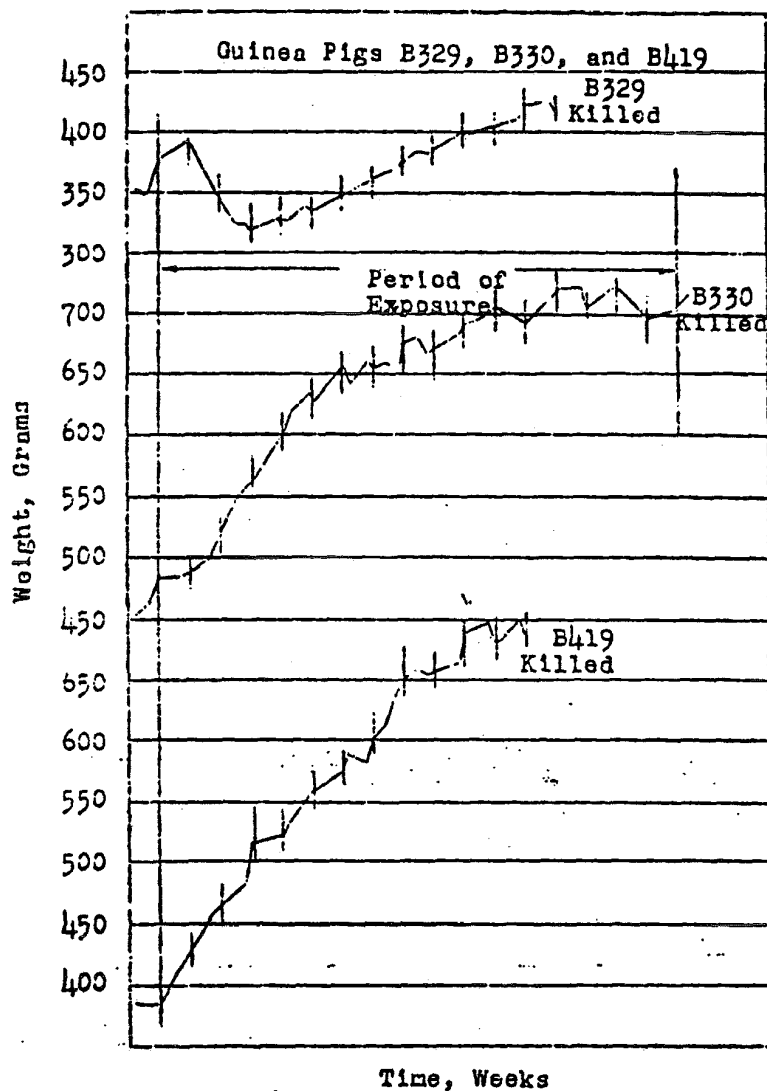
NEV 016621





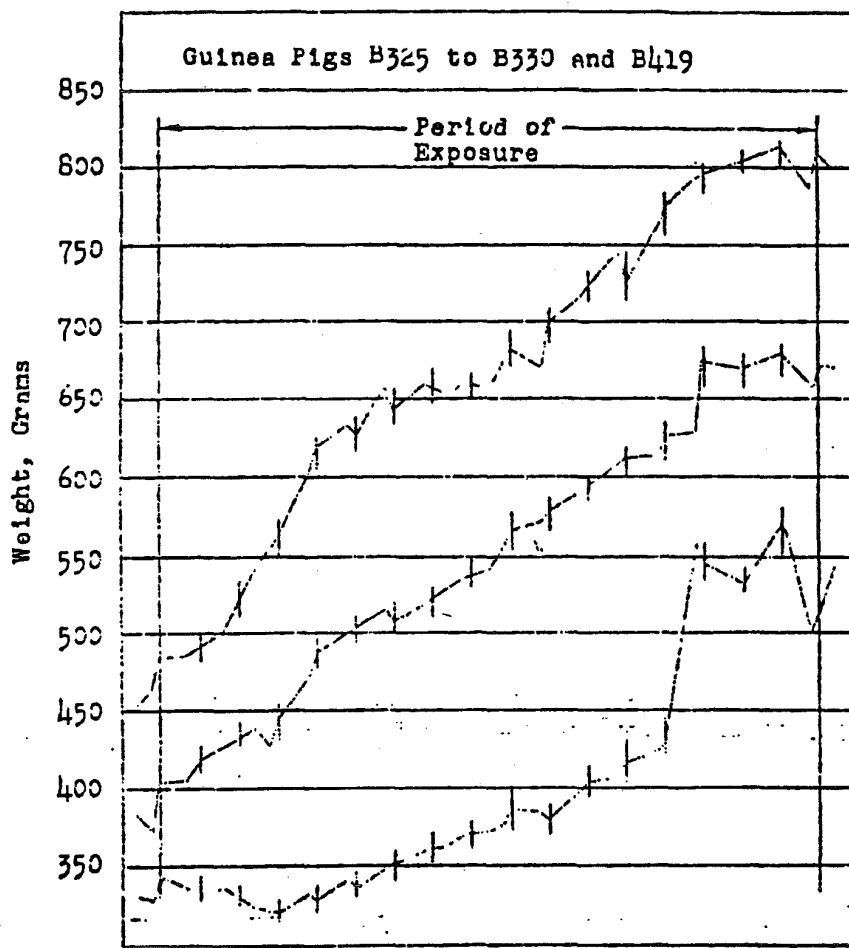
Experiment No. 2
 6.8 Micrograms of Aroclor 1242/liter
 Figure 11

NEV 016623



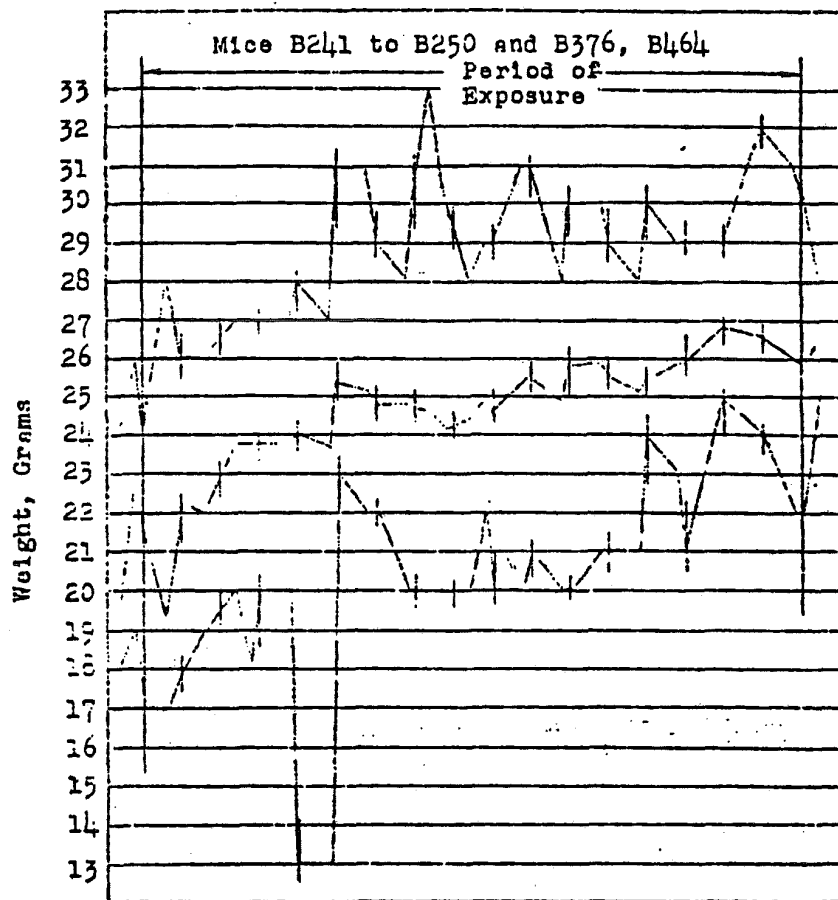
Experiment No. 2
6.8 Micrograms of Aroclor 1242/liter
Figure 12

NEV 016024



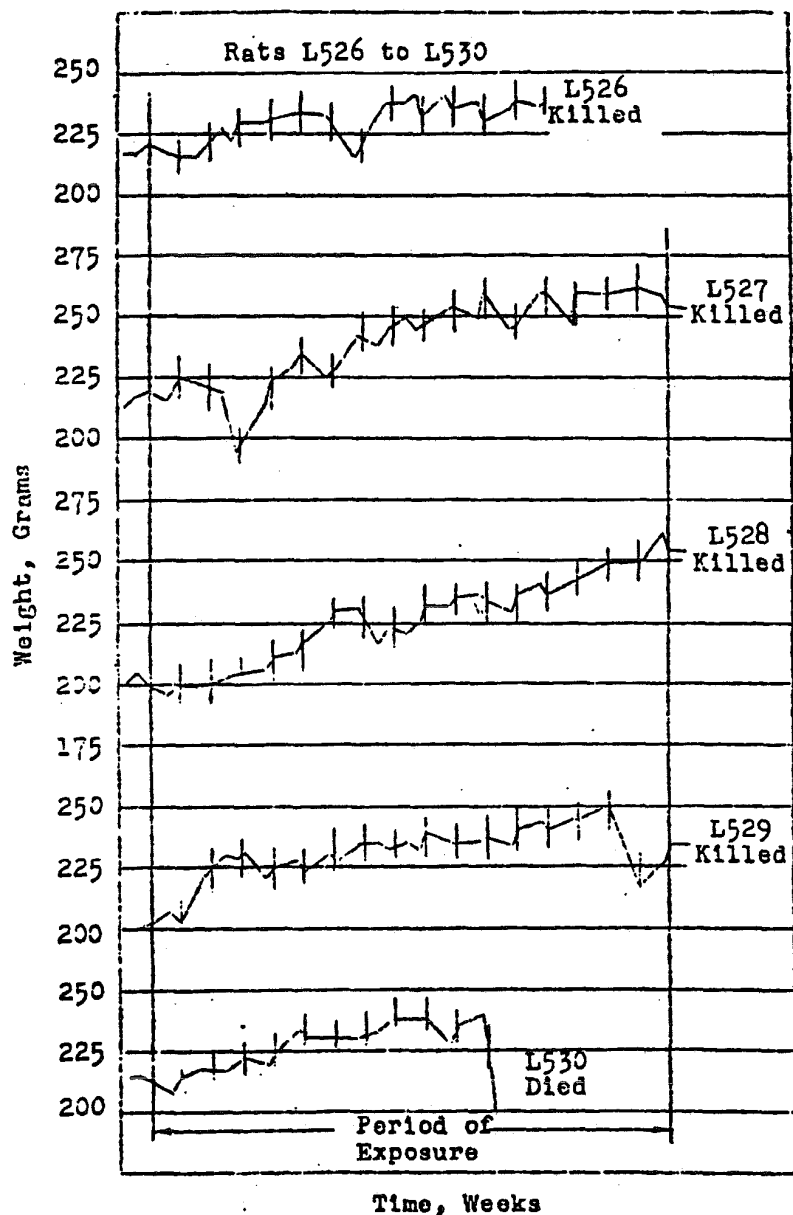
Experiment No. 2
6.8 Micrograms of Aroclor 1242/liter
Figure 13

NEV 016625



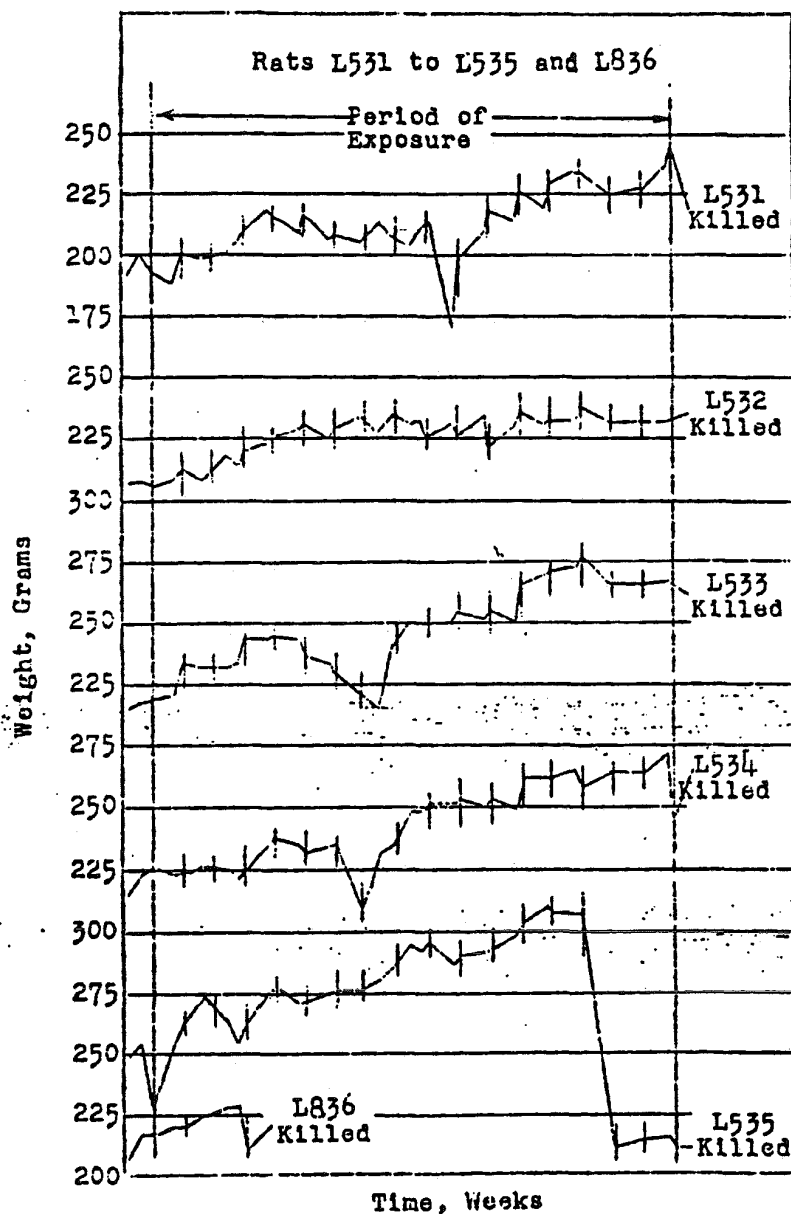
Experiment No. 2
 6.8 Micrograms of Aroclor 1242/liter
 Figure 14

NEV 016626



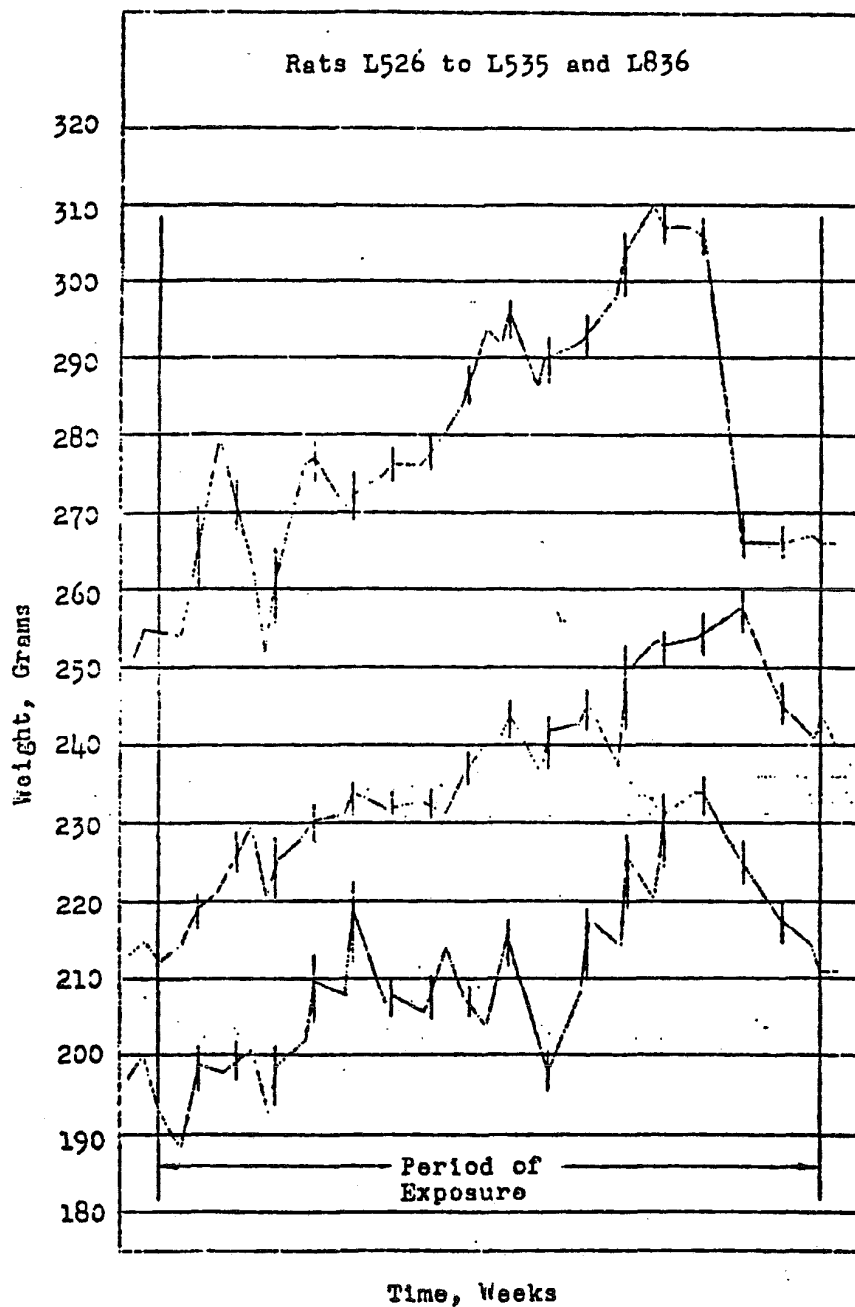
Experiment No. 2
6.8 Micrograms of Aroclor 1242/liter
Figure 15

NEV 016627



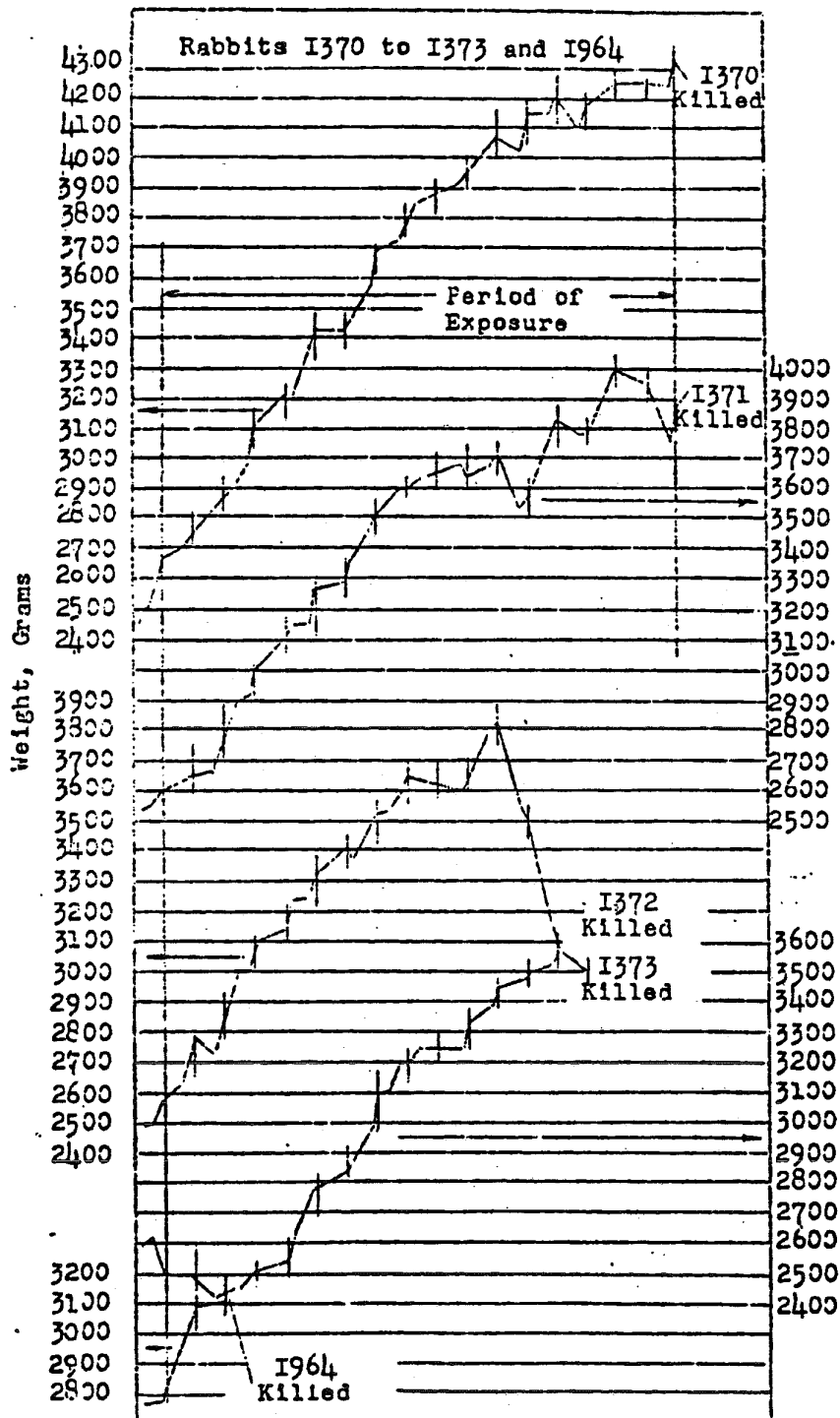
Experiment No. 2
6.8 Micrograms of Aroclor 1242/liter
Figure 16

NEV 016028



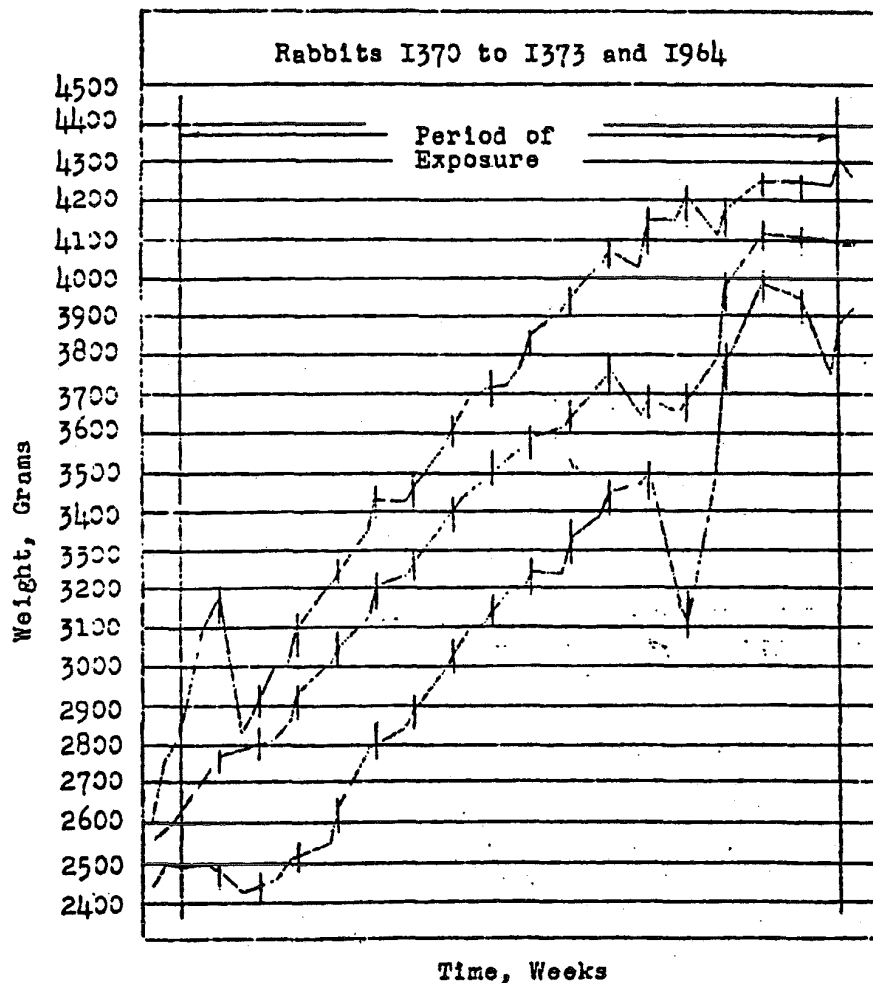
Experiment No. 2
 6.8 Micrograms of Aroclor 1242/liter
 Figure 17

NEV 016629



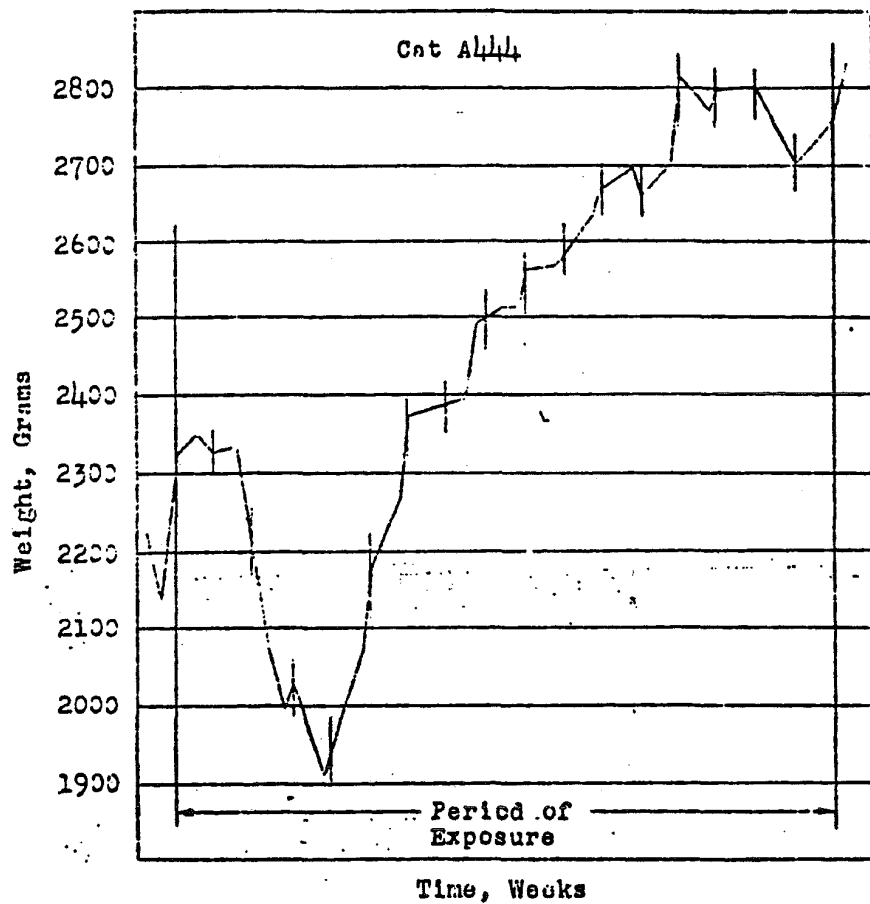
Experiment No. 2
6.8 Micrograms of Aroclor 1242/liter
Figure 18

NEW 016630



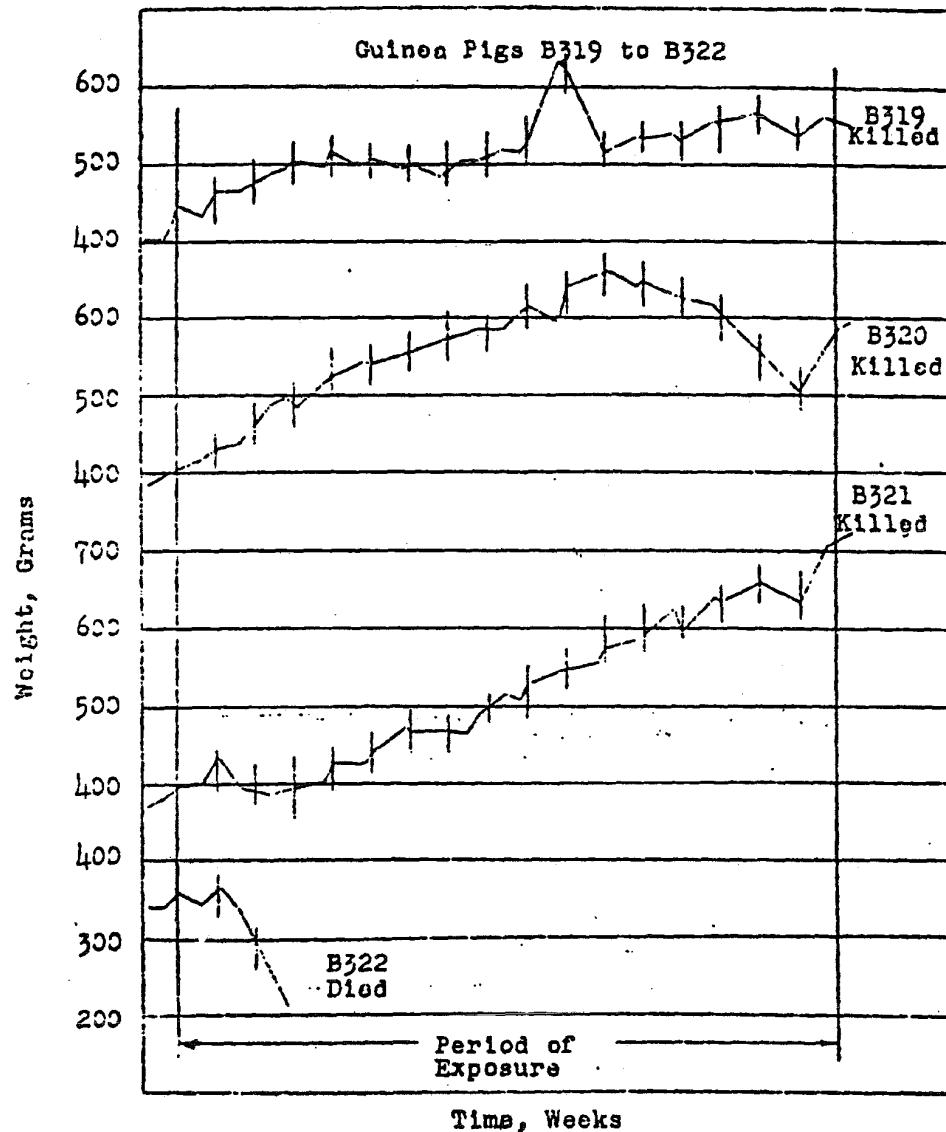
Experiment No. 2
6.8 Micrograms of Aroclor 1242/liter
Figure 19

NEV 016031



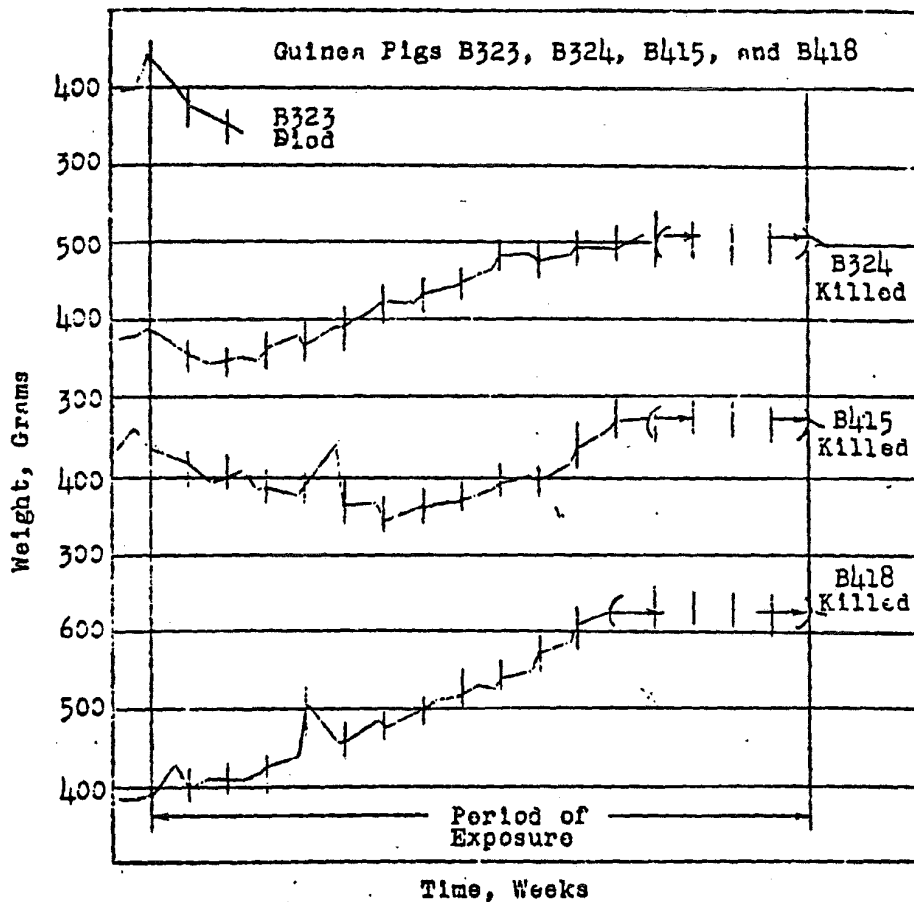
Experiment No. 1
5.4 Micrograms of Aroclor 1254/liter
Figure 20

NEV 016632



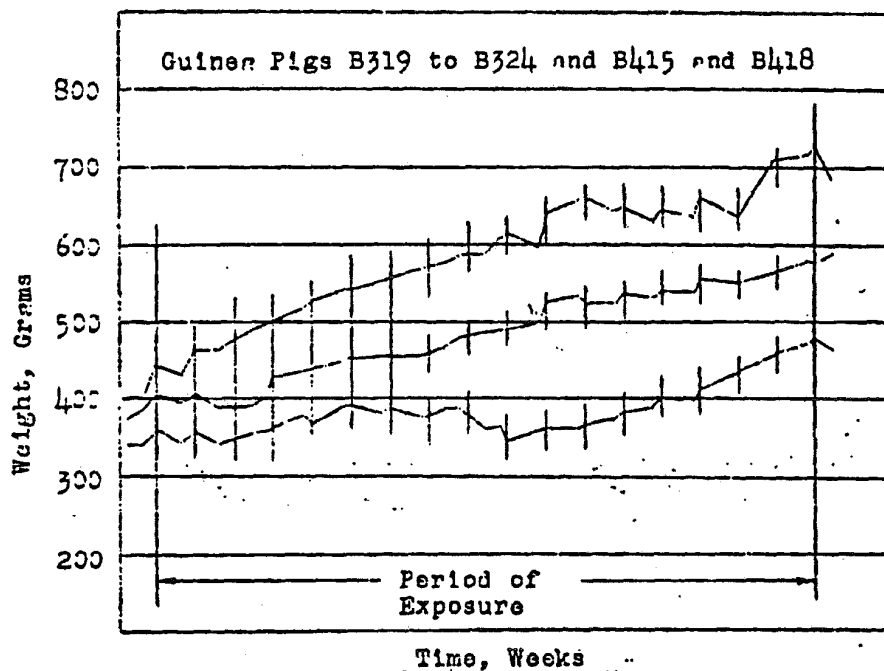
Experiment No. 1
 5.4 Micrograms of Aroclor 1254/liter
 Figure 21

NEV 016633



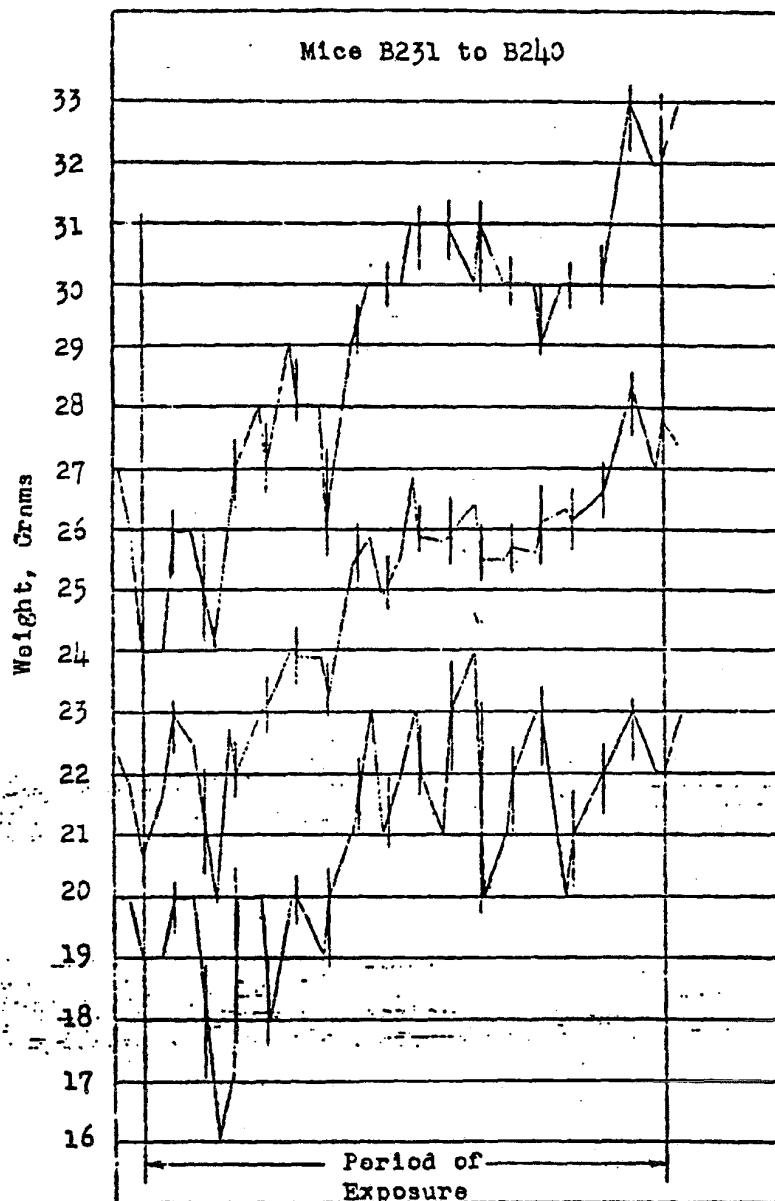
Experiment No. 1
 5.4 Micrograms of Aroclor 1254/liter
 Figure 22

NEV 016634



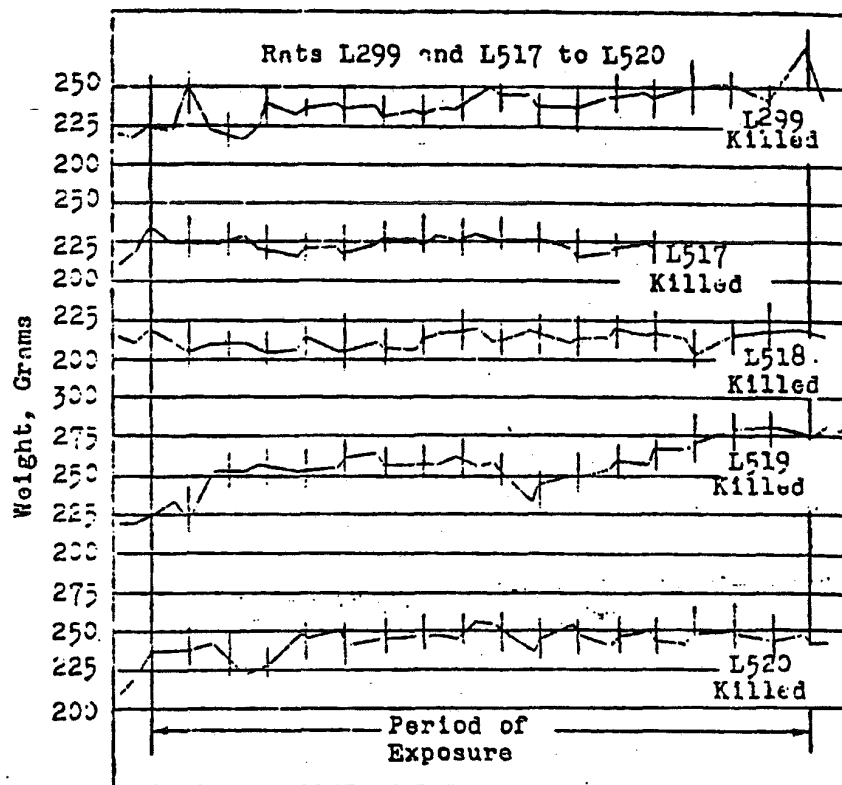
Experiment No. 1
 5.4 Micrograms of Aroclor 1254/liter
 Figure 23

NEV 016635



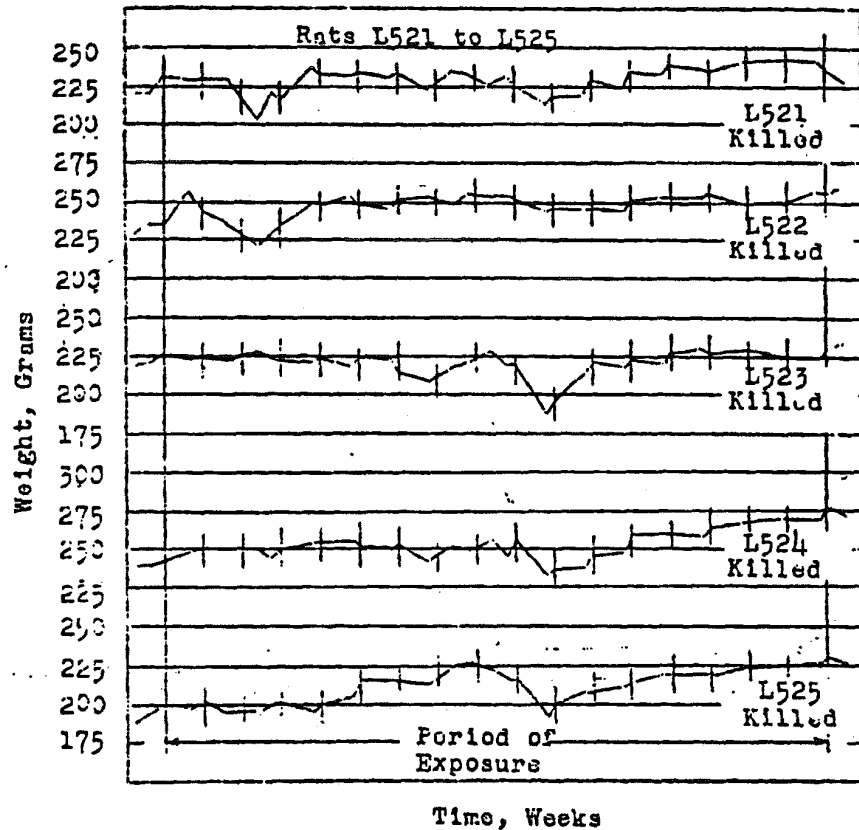
Experiment No. 1
 5.4 Micrograms of Aroclor 1254/liter
 Figure 24

NEV 016636



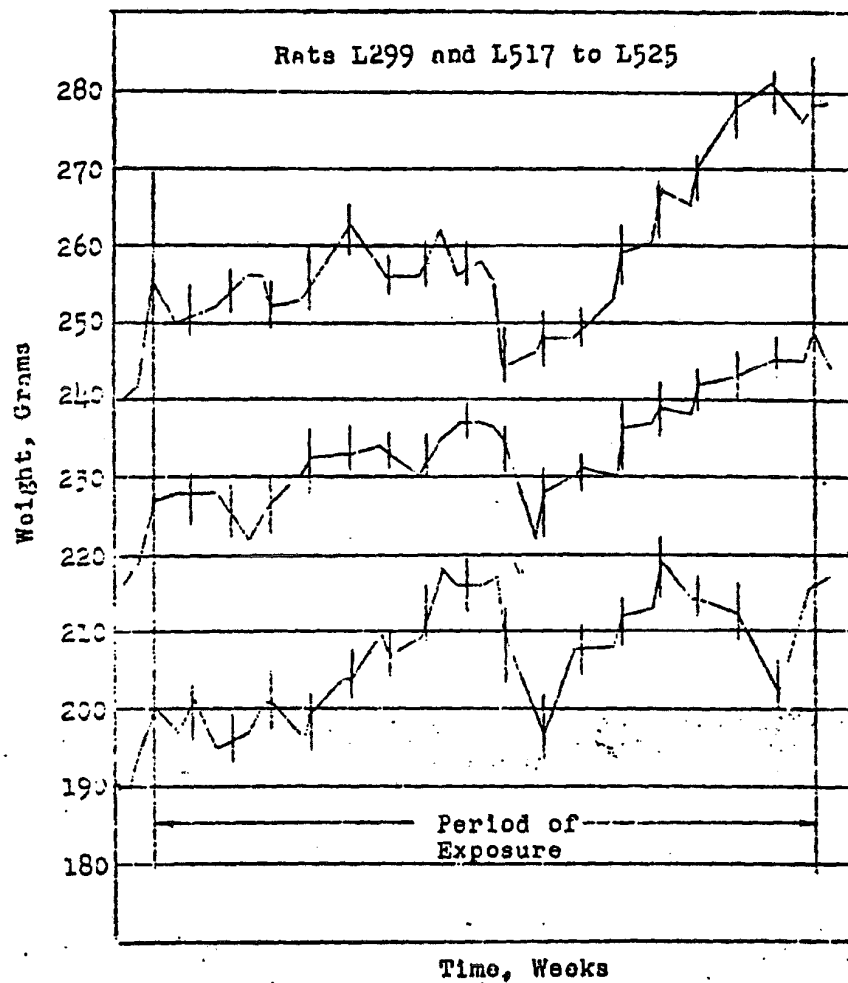
Experiment No. 1
 5.4 Micrograms of Aroclor 1254/liter
 Figure 25

NEV 016637



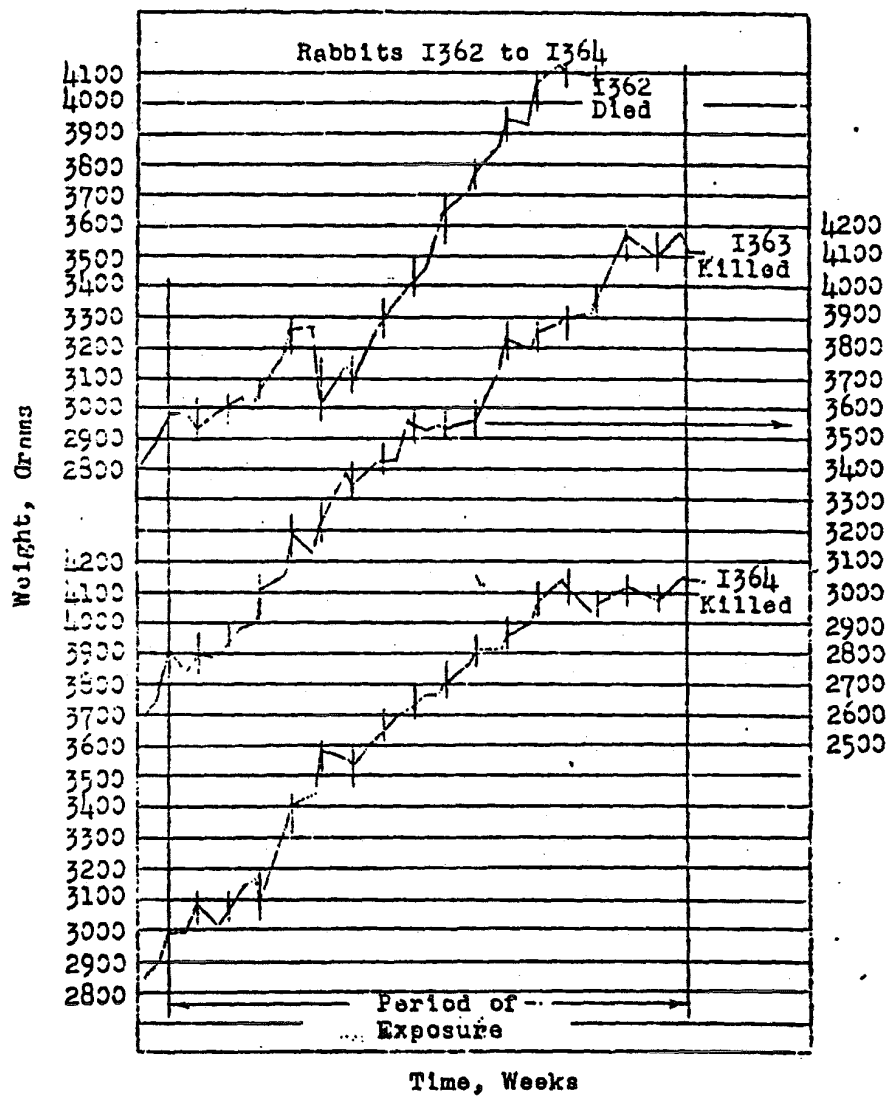
Experiment No. 1
 5.4 Micrograms of Aroclor 1254/liter
 Figure 26

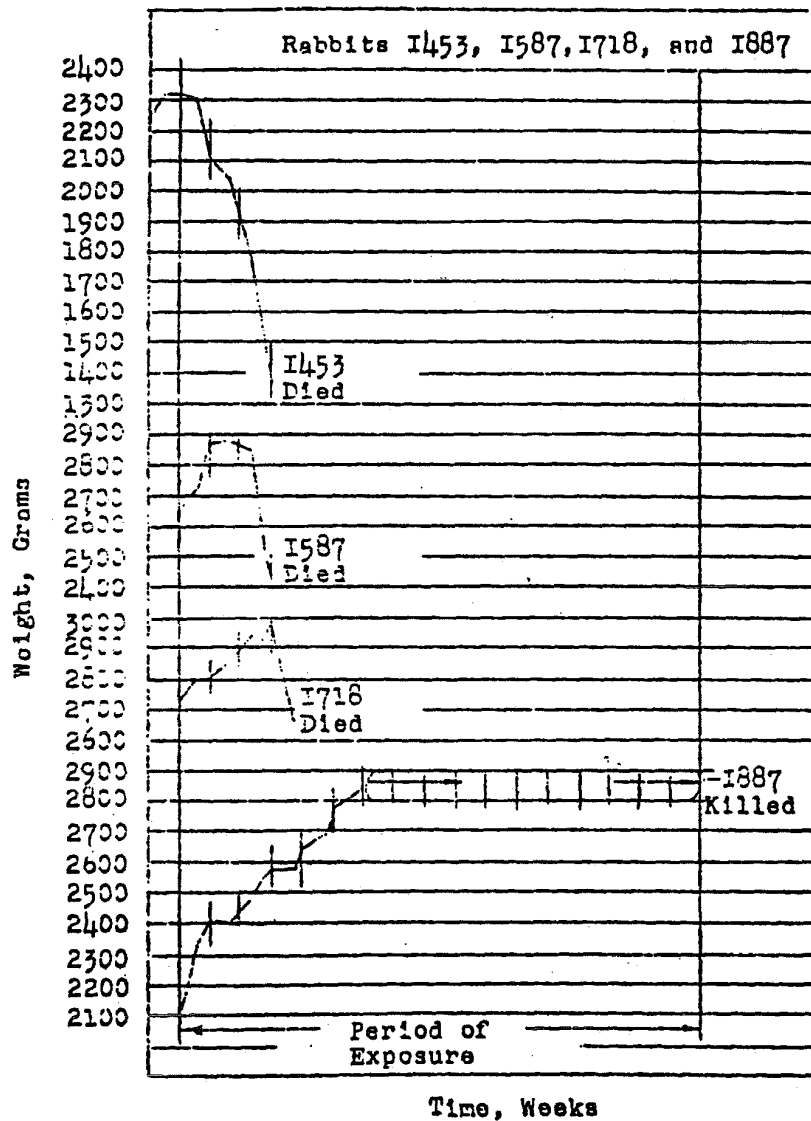
NEV 016638



Experiment No. 1
 5.4 Micrograms of Aroclor 1254/liter
 Figure 27

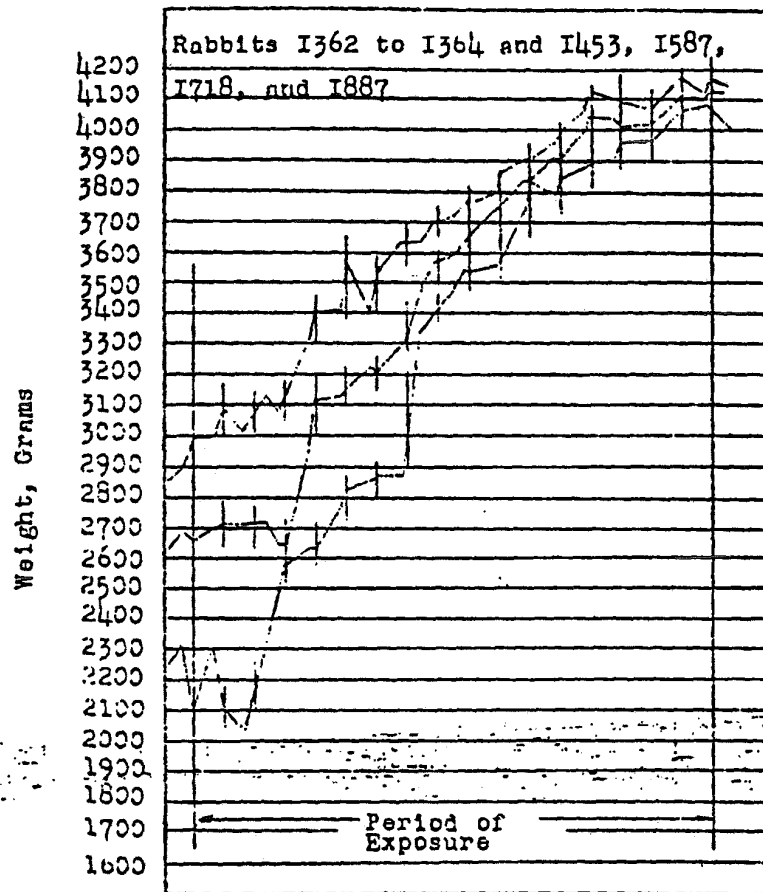
NEV 016639





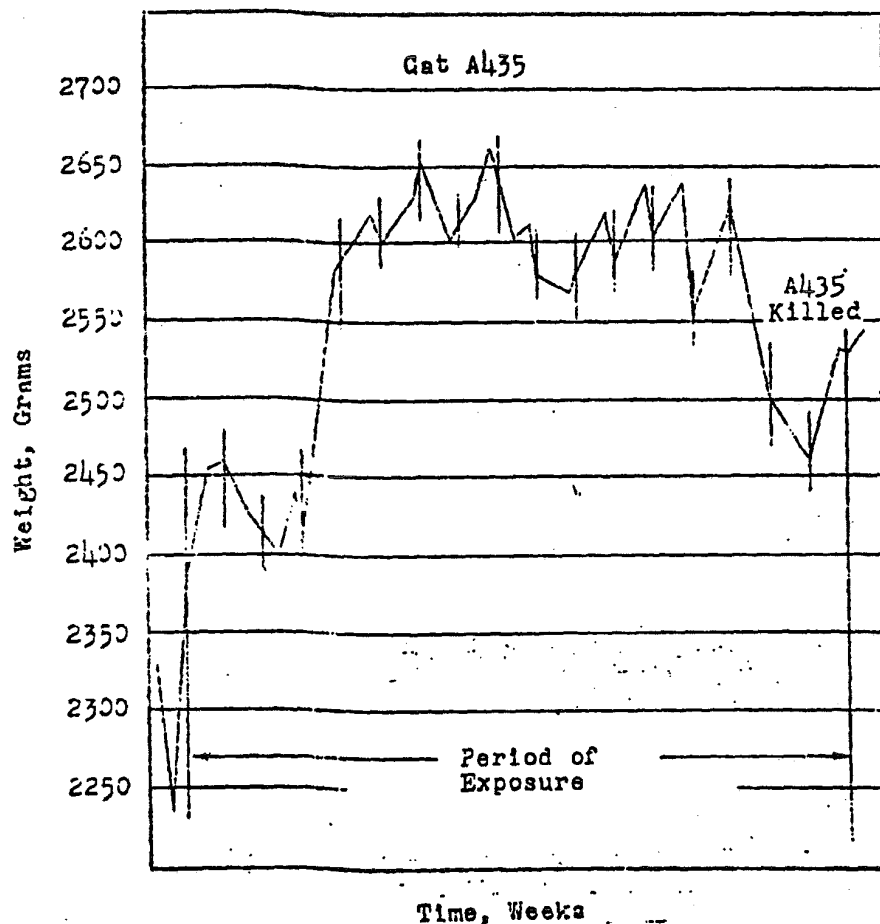
Experiment No. 1
 5.4 Micrograms of Aroclor 1254/liter
 Figure 29

NEV 016641



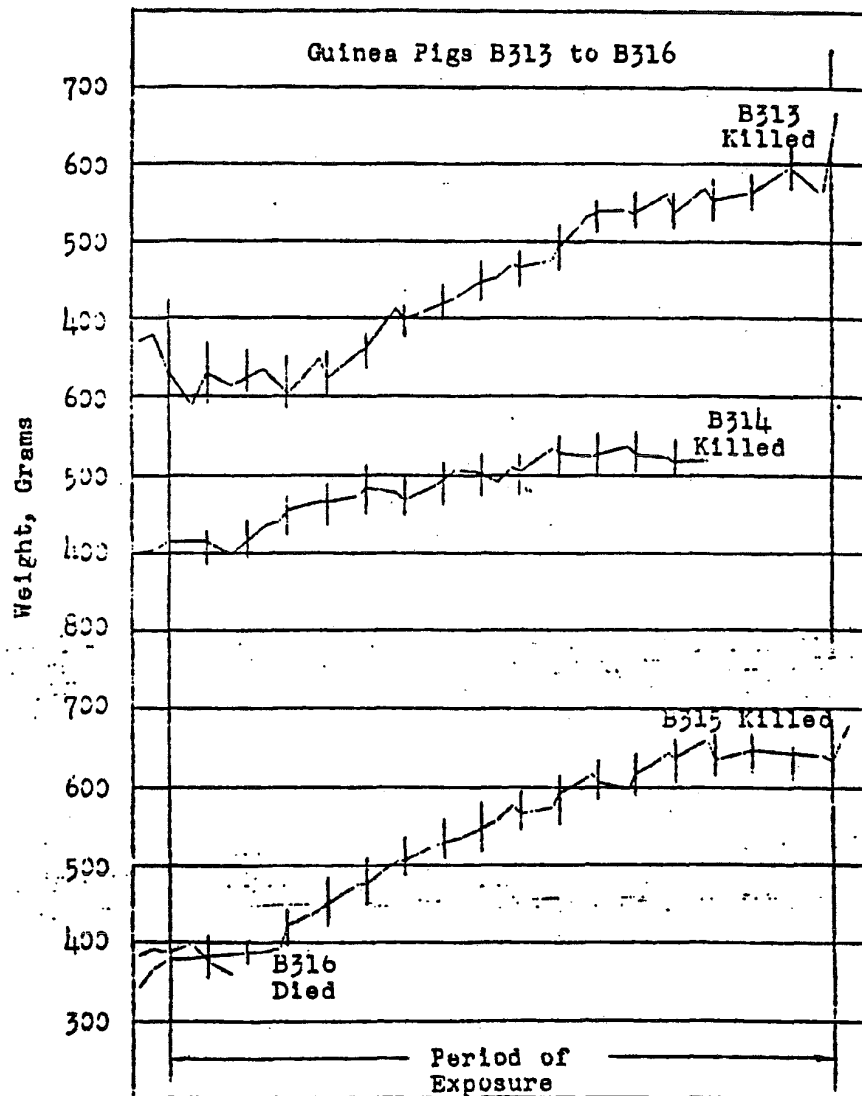
Experiment No. 1
5.4 Micrograms of Aroclor 1254/liter
Figure 30

NEV 016642



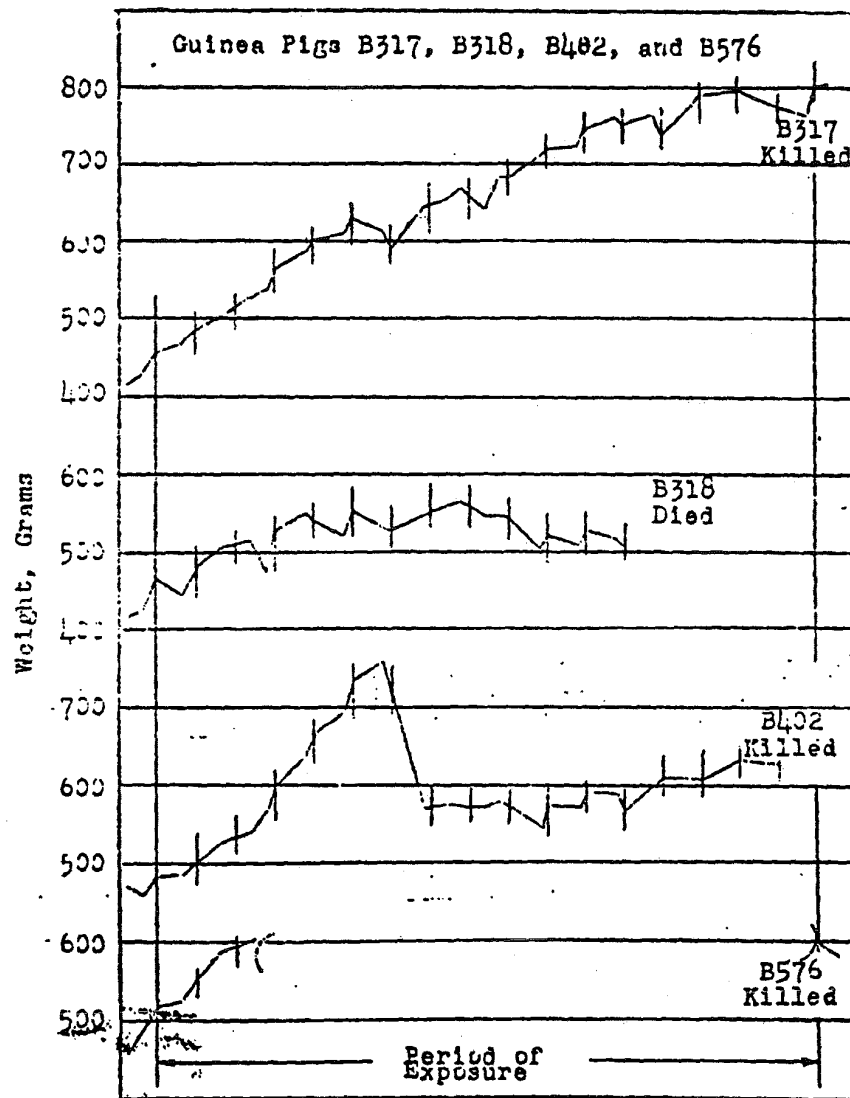
Control Subjected to Conditioned Air; for
 Experiment No. 2 Aroclor 1242 and
 Experiment No. 1 Aroclor 1254
 Figure 31

NEV 016643



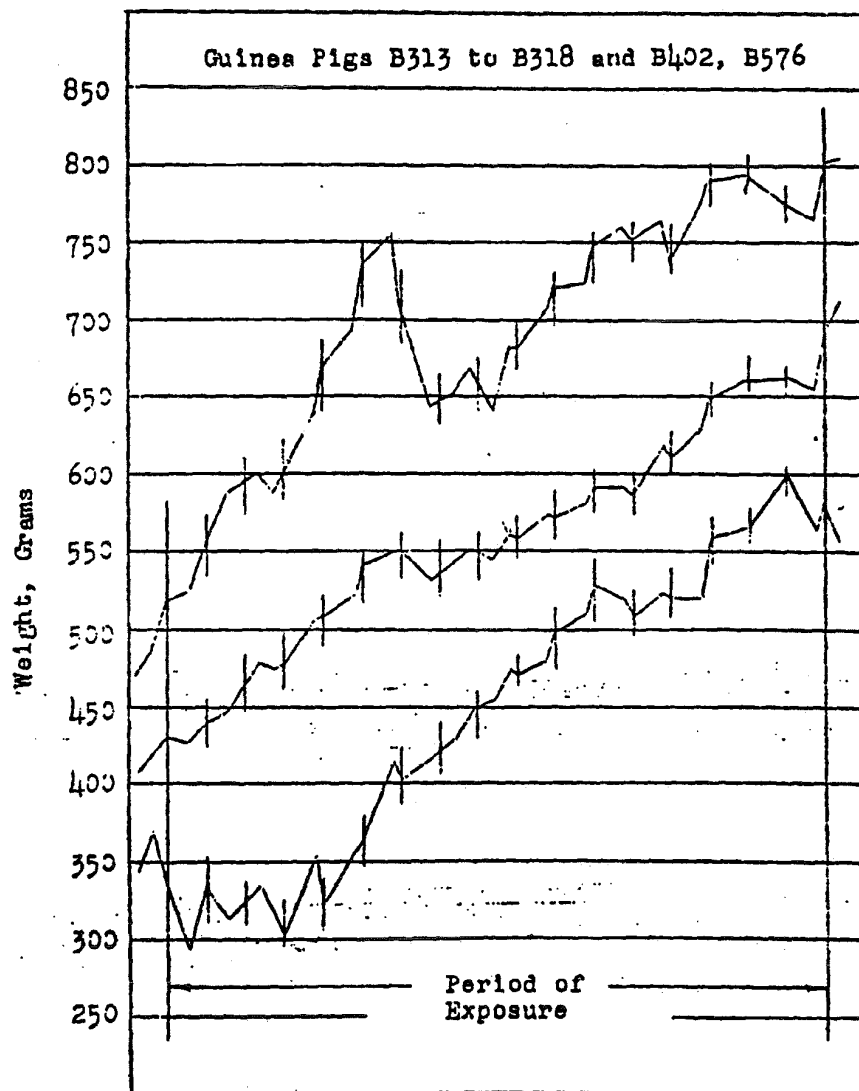
Controls Subjected to Conditioned Air; for
 Experiment No. 2 Aroclor 1242 and
 Experiment No. 1 Aroclor 1254
 Figure 32

NEV 016644



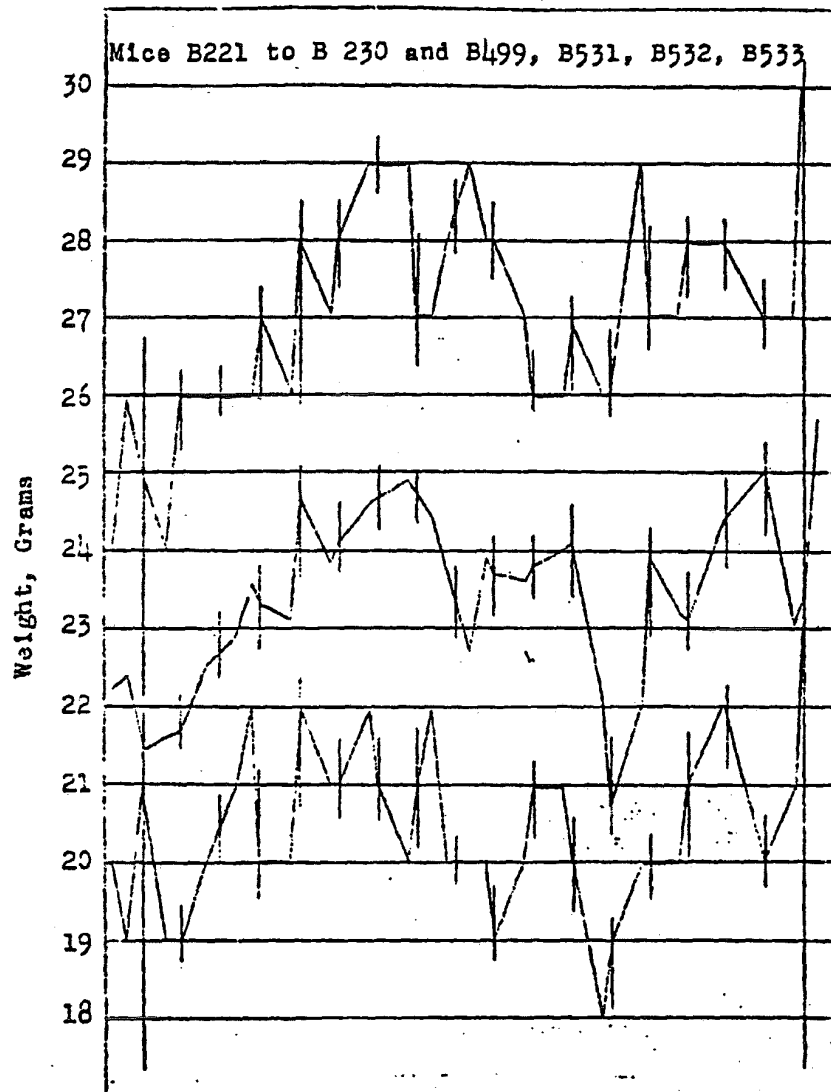
Time, Weeks
 Controls Subjected to Conditioned Air; for
 Experiment No. 2 Aroclor 1242 and
 Experiment No. 1 Aroclor 1254
 Figure 33

NEV 016645



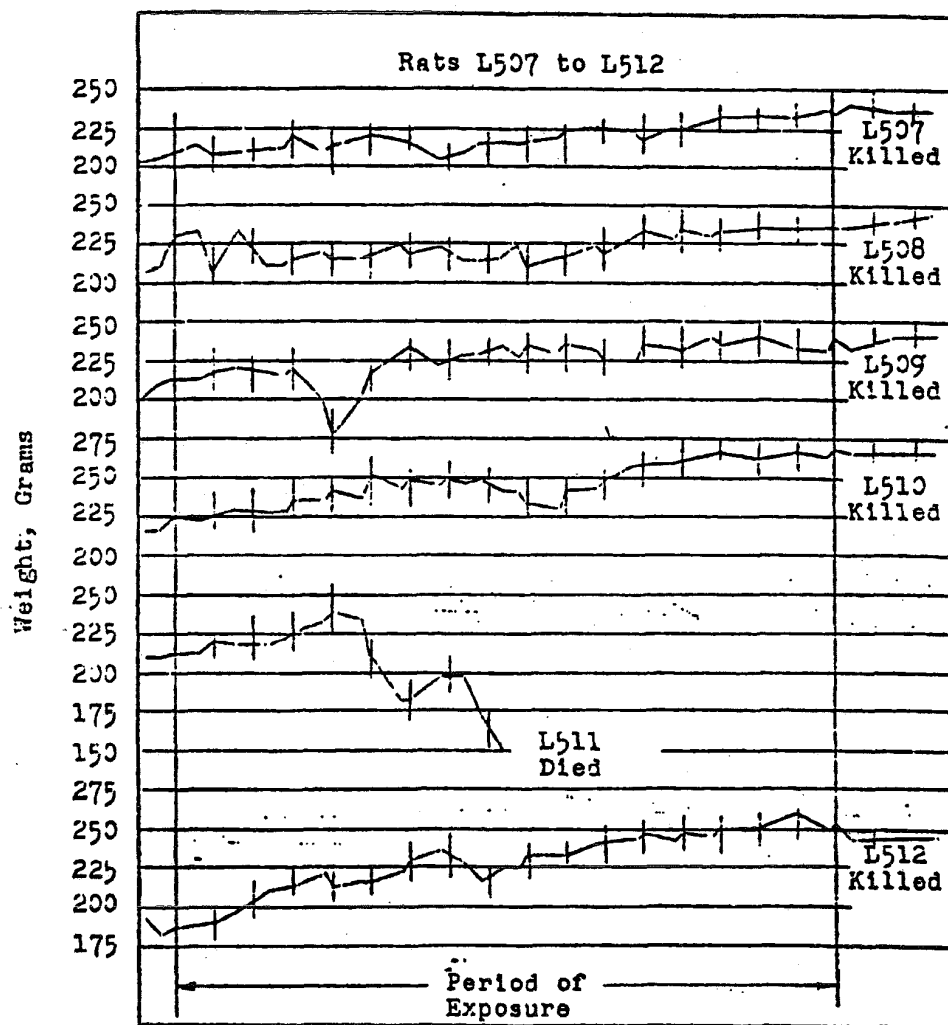
Time, Weeks
 Controls Subjected to Conditioned Air; for
 Experiment No. 2 Aroclor 1242 and
 Experiment No. 1 Aroclor 1254
 Figure 34

NEV 016646



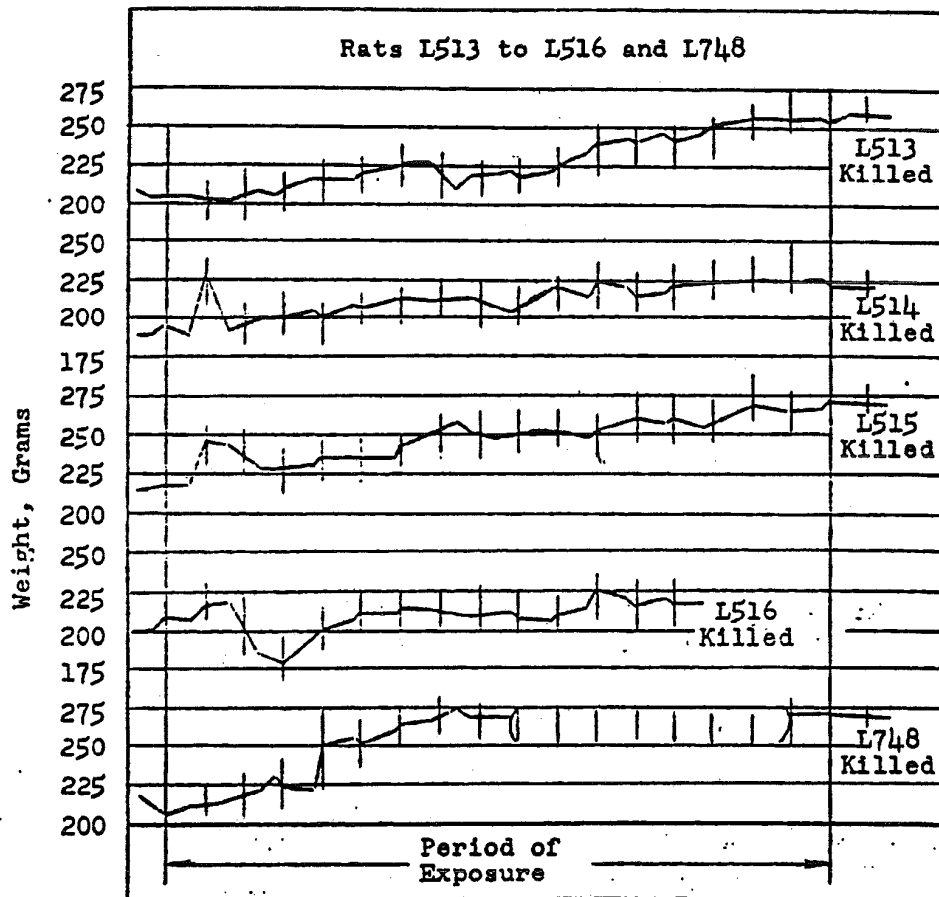
Controls Subjected to Conditioned Air; for
 Experiment No. 2 Aroclor 1242 and
 Experiment No. 1 Aroclor 1254
 Figure 35

NEV 016647



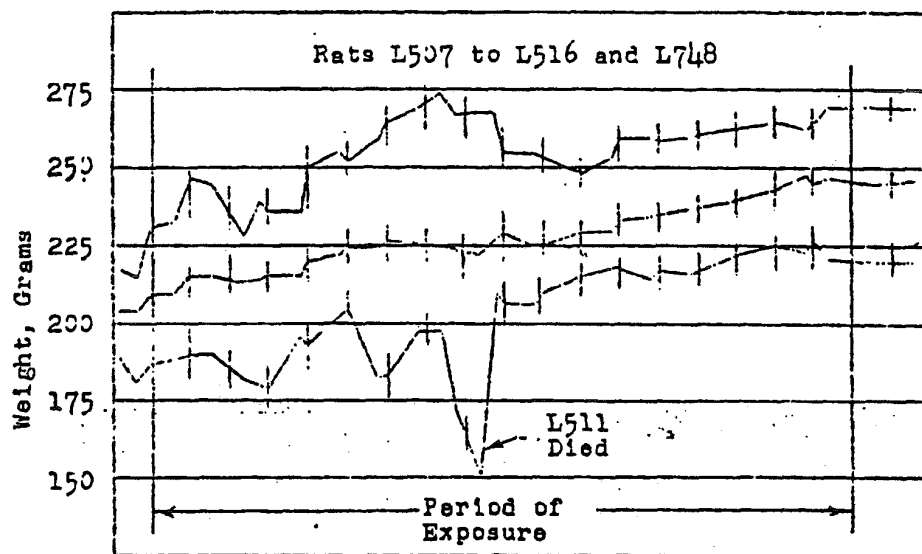
Controls Subjected to Conditioned Air; for
 Experiment No. 2 Aroclor 1242 and
 Experiment No. 1 Aroclor 1254
 Figure 36

NEV 016648



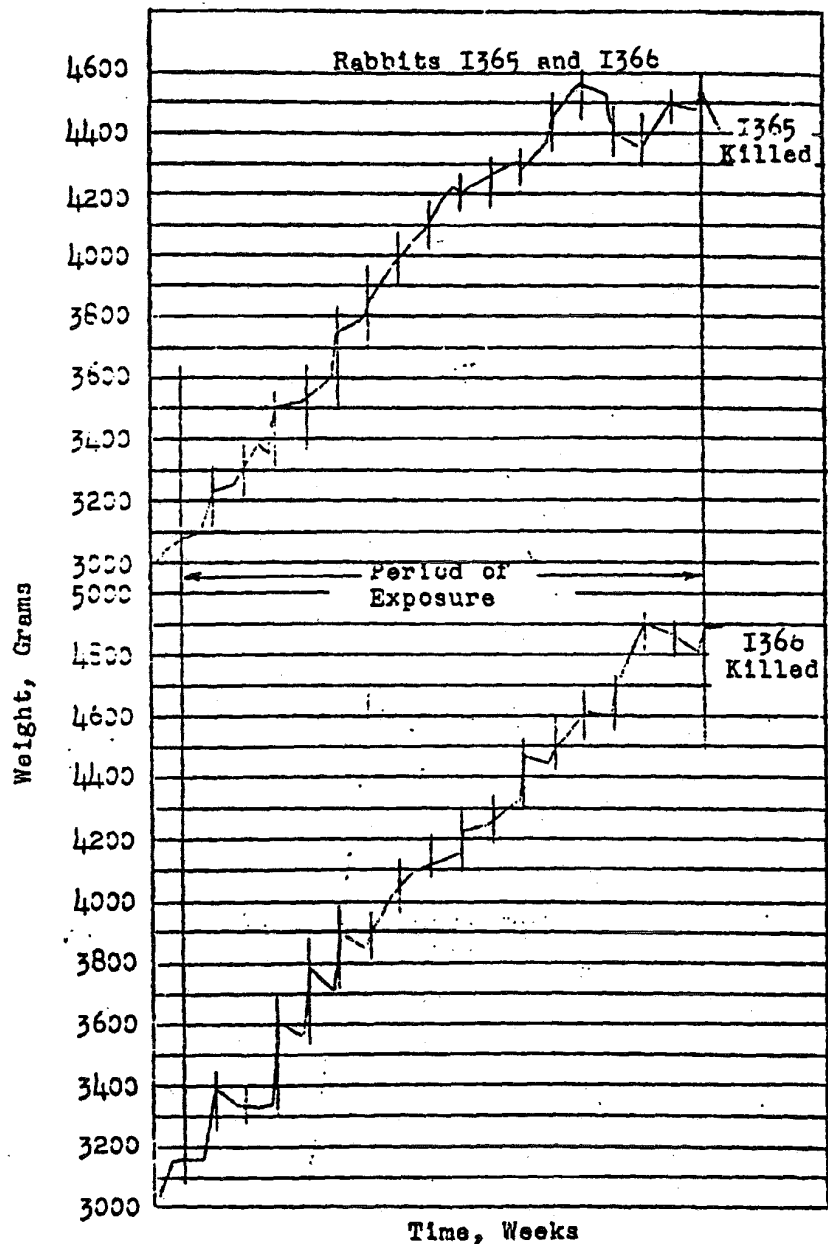
Time, Weeks
 Controls Subjected to Conditioned Air; for
 Experiment No. 2 Aroclor 1242 and
 Experiment No. 1 Aroclor 1254
 Figure 37

NEV 016649



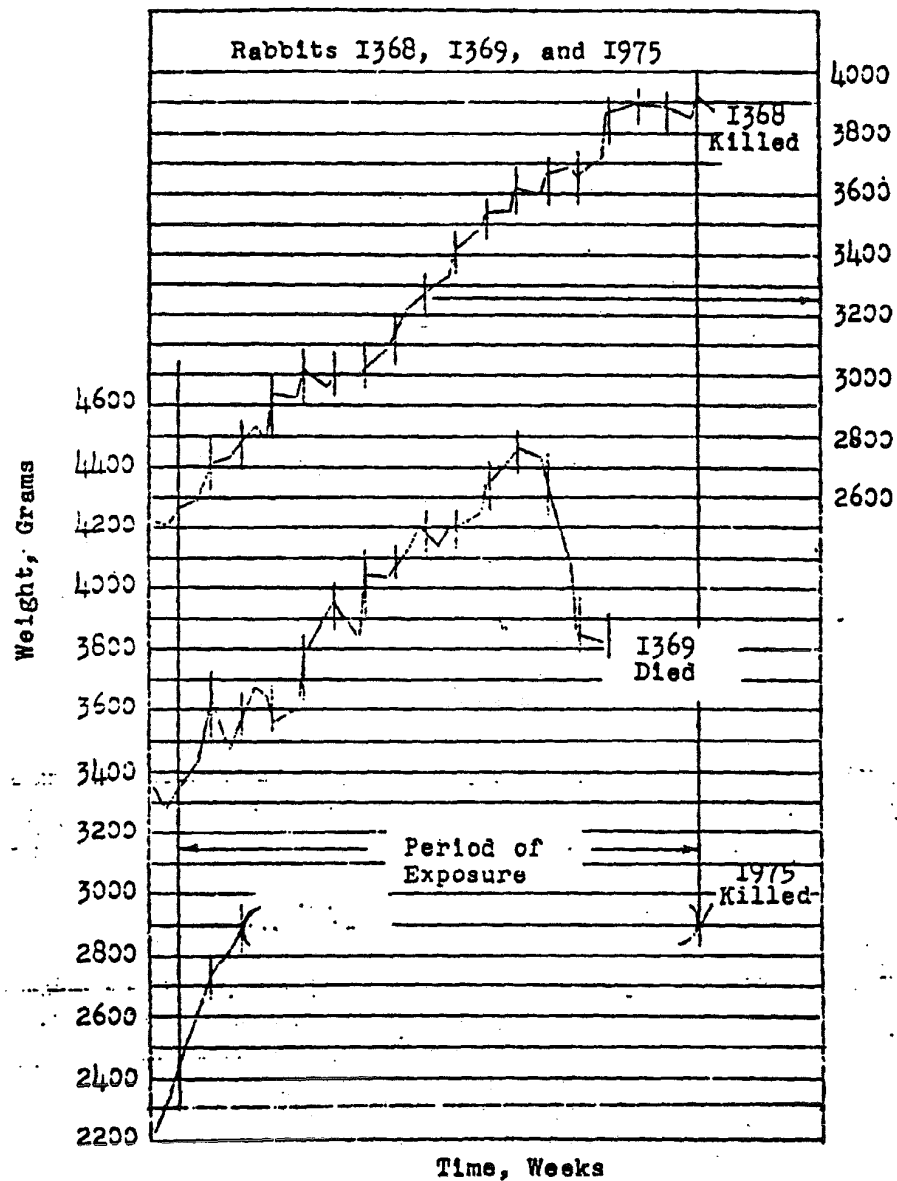
Controls Subjected to Conditioned Air; for
 Experiment No. 2 Aroclor 1242 and
 Experiment No. 1 Aroclor 1254
 Figure 38

NEV 016650



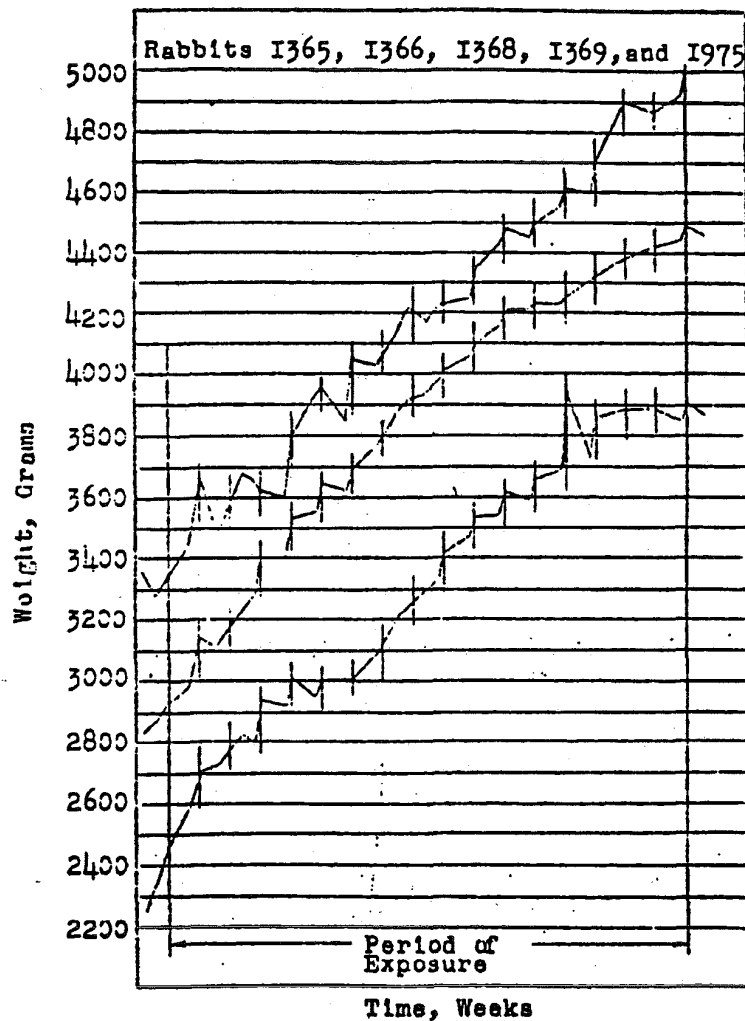
Time, Weeks
 Controls Subjected to Conditioned Air; for
 Experiment No. 2 Aroclor 1242 and
 Experiment No. 1 Aroclor 1254
 Figure 39

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Time, Weeks
 Controls Subjected to Conditioned Air; for
 Experiment No. 2 Aroclor 1242 and
 Experiment No. 1 Aroclor 1254
 Figure 40

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Controls Subjected to Conditioned Air; for
 Experiment No. 2 Aroclor 1242 and
 Experiment No. 1 Aroclor 1254
 Figure 41

NEV 016653

K-34-050

The Toxicity of the Vapor of
Aroclor 1242 and of Aroclor 1254

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DEPOSITION
EXHIBIT

13

2-1594

June 28, 1955

THE KETTERING LABORATORY

in the

Department of Preventive Medicine and Industrial Health

College of Medicine

UNIVERSITY OF CINCINNATI, CINCINNATI, OHIO

NEV 016654

Sequence of Tables

1. The Mortality, Length of Survival, and Changes in Weight of Animals Exposed to the Vapor of Aroclor 1242 in Air.
2. The Mortality, Length of Survival, and Changes in Weight of Animals Exposed to the Vapor of Aroclor 1254 in Air.
3. The Mortality, Length of Survival, and Changes in Weight of Animals Exposed to Conditioned Air.
4. Summary of the Data on the Mortality Among Experimental and Control Animals.
5. The Average Changes in Weight of the Survivors Among the Original Groups.

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Sequence of Figures

1. Weight of Cat A-499 Recorded at Weekly Intervals During the Period of Exposure to the Vapor of Aroclor 1242 in Air - 1.9 micrograms per liter (Experiment No. 3).
2. Weights of Individual Guinea Pigs B-599, B-600, B-601 and B-828 Recorded at Weekly Intervals During the Period of Exposure to the Vapor of Aroclor 1242 in Air - 1.9 micrograms per liter (Experiment No. 3).
3. Weights of Individual Guinea Pigs B-602, B-603 and B-604 Recorded at Weekly Intervals During the Period of Exposure to the Vapor of Aroclor 1242 in Air - 1.9 micrograms per liter (Experiment No. 3).
4. Average and Range of Weight Recorded at Weekly Intervals of Guinea Pigs B-599 and B-601 to B-604 that Survived throughout the Period of Exposure to the Vapor of Aroclor 1242 in Air - 1.9 micrograms per liter (Experiment No. 3).
5. Weights of Individual Mice B-567, B-568, B-569 and B-600 Recorded at Weekly Intervals During the Period of Exposure to the Vapor of Aroclor 1242 in Air - 1.9 micrograms per liter (Experiment No. 3).
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8. Average and Range of Weight Recorded at Weekly Intervals of Mice B-567, B-568, B-571, B-572, B-573 and B-574 that Survived throughout the Period of Exposure to the Vapor of Aroclor 1242 in Air - 1.9 micrograms per liter (Experiment No. 3).

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9. Weights of Individual Rabbits H-1061, H-1062, J-784, J-822, J-828, J-861, J-863, J-864 and J-886 Recorded at Weekly Intervals During the Period of Exposure to the Vapor of Aroclor 1242 in Air - 1.9 micrograms per liter (Experiment No. 3).
10. Weights of Individual Rabbits H-1063, H-1064, J-84, J-324, J-760, J-823, J-862, J-885 and J-887 Recorded at Weekly Intervals During the Period of Exposure to the Vapor of Aroclor 1242 in Air - 1.9 micrograms per liter (Experiment No. 3).
11. Weights of Individual Rats L-992, L-993 and L-994 Recorded at Weekly Intervals During the Period of Exposure to the Vapor of Aroclor 1242 in Air - 1.9 micrograms per liter (Experiment No. 3).
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13. Weights of Individual Rats L-998, L-999, L-1000, M-1 and M-445 Recorded at Weekly Intervals During the Period of Exposure to the Vapor of Aroclor 1242 in Air - 1.9 micrograms per liter (Experiment No. 3).
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15. Weight of Cat A-500 Recorded at Weekly Intervals During the Period of Exposure to the Vapor of Aroclor 1254 in Air - 1.5 micrograms per liter (Experiment No. 2).
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18. Average and Range of Weight Recorded at Weekly Intervals of Guinea Pigs B-606 and B-608 to B-610 that Survived throughout the Period of Exposure to the Vapor of Aroclor 1254 in Air - 1.5 micrograms per liter (Experiment No. 2).
19. Weights of Individual Mice B-577, B-578 and B-579 Recorded at Weekly Intervals During the Period of Exposure to the Vapor of Aroclor 1254 in Air - 1.5 micrograms per liter (Experiment No. 2).
20. Weights of Individual Mice B-580, B-581, B-582 and B-919 Recorded at Weekly Intervals During the Period of Exposure to the Vapor of Aroclor 1254 in Air - 1.5 micrograms per liter (Experiment No. 2).
21. Weights of Individual Mice B-583, B-584, B-585, B-586, B-602, B-603, B-673 and B-863 Recorded at Weekly Intervals During the Period of Exposure to the Vapor of Aroclor 1254 in Air - 1.5 micrograms per liter (Experiment No. 2).
22. Average and Range of Weight Recorded at Weekly Intervals of Mice B-577 to B-580, B-582 and B-585 that Survived throughout the Period of Exposure to the Vapor of Aroclor 1254 in Air - 1.5 micrograms per liter (Experiment No. 2).
23. Weights of Individual Rabbits H-1065 and H-1066 Recorded at Weekly Intervals During the Period of Exposure to the Vapor of Aroclor 1254 in Air - 1.5 micrograms per liter (Experiment No. 2).
24. Weights of Individual Rabbits H-1067 and H-1068 Recorded at Weekly Intervals During the Period of Exposure to the Vapor of Aroclor 1254 in Air - 1.5 micrograms per liter (Experiment No. 2).
25. Average and Range of Weight Recorded at Weekly Intervals of Rabbits H-1065 to H-1068 that Survived throughout the Period of Exposure to the Vapor of Aroclor 1254 in Air - 1.5 micrograms per liter (Experiment No. 2).
26. Weights of Individual Rats M-2, M-3 and M-4 Recorded at Weekly Intervals During the Period of Exposure to the Vapor of Aroclor 1254 in Air - 1.5 micrograms per liter (Experiment No. 2).

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27. Weights of Individual Rats M-5, M-6, M-7 and M-8 Recorded at Weekly Intervals During the Period of Exposure to the Vapor of Aroclor 1254 in Air - 1.5 micrograms per liter (Experiment No. 2).
 28. Weights of Individual Rats M-9, M-10, M-11 and M-140. Recorded at Weekly Intervals During the Period of Exposure to the Vapor of Aroclor 1254 in Air - 1.5 micrograms per liter (Experiment No. 2).
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 30. Weight of Control Cat A-510 Recorded at Weekly Intervals During the Period of Confinement in Conditioned Air (Experiment No. 2).
 31. Weights of Individual Control Guinea Pigs B-611, B-612, B-613 and B-798 Recorded at Weekly Intervals During the Period of Confinement in Conditioned Air (Experiment No. 2).
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 34. Weights of Individual Control Mice B-587, B-588, B-589, B-968 and B-969 Recorded at Weekly Intervals During the Period of Confinement in Conditioned Air (Experiment No. 2).
 35. Weights of Individual Control Mice B-590, B-591, B-592, B-864, B-936 and B-967 Recorded at Weekly Intervals During the Period of Confinement in Conditioned Air (Experiment No. 2).
 36. Weights of Individual Control Mice B-593, B-594, B-595, B-596, B-933, B-935 and B-970 Recorded at Weekly Intervals During the Period of Confinement in Conditioned Air (Experiment No. 2).

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37. Average and Range of Weight Recorded at Weekly Intervals of Control Mice B-588, B-589, B-591 and B-594 to B-596 that Survived throughout the Period of Confinement of Conditioned Air (Experiment No. 2).
38. Weights of Individual Control Rabbits H-1069 and H-1070 Recorded at Weekly Intervals During the Period of Confinement in Conditioned Air (Experiment No. 2).
39. Weights of Individual Control Rabbits H-1071, H-1072, J-183 and J-202 Recorded at Weekly Intervals During the Period of Confinement in Conditioned Air (Experiment No. 2).
40. Average and Range of Weight Recorded at Weekly Intervals of Control Rabbits H-1069, H-1070, J-183 and J-202 During the Period of Confinement in Conditioned Air (Experiment No. 2).
41. Weights of Individual Control Rats M-12, M-13 and M-14 Recorded at Weekly Intervals During the Period of Confinement in Conditioned Air (Experiment No. 2).
42. Weights of Individual Control Rats M-15, M-16 and M-17 Recorded at Weekly Intervals During the Period of Confinement in Conditioned Air (Experiment No. 2).
43. Weights of Individual Control Rats M-18, M-19, M-20, M-21 and M-351 Recorded at Weekly Intervals During the Period of Confinement in Conditioned Air (Experiment No. 2).
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The Toxicity of the Vapor of
Aroclor 1242 and of Aroclor 1254

(Supplement to Report of June 22, 1955)

Scope

This supplemental report presents the results obtained when separate groups of animals were exposed, respectively, to Aroclor 1242 and Aroclor 1254 in lower concentrations in the atmosphere and for a longer period than were those employed in the experiments described in the report of June 22, 1955. Since these experiments involved a different period of time and a different season of the year, a new group of control animals was included.

Summary of Results

1. In an experiment in which, for 7 hours per day on 150 days over a period of 214 days, animals were exposed to air bearing Aroclor 1242 in the concentration of 1.9 micrograms per liter (0.15 ppm), the incidence of mortality among the various species, with the exception of the rabbits (which died during an epidemic of pulmonary disease), was no greater than that encountered among a similarly constituted control group. The growth of the experimental animals was unaffected.

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No gross or microscopic evidence of general or specific injury was found in the tissues of the animals as a response to their prolonged exposure to Aroclor 1242.

2. In another experiment, in which animals were exposed to air bearing Aroclor 1254 in the concentration of 1.5 micrograms per liter (0.11 ppm), for 7 hours per day on 150 days over a period of 213 days, the incidence of mortality was no greater than that observed among corresponding groups of controls. The growth of the experimental groups did not differ significantly from that of the controls. No relevant gross pathological changes were observed in the animals, but microscopic lesions of a mild, nonspecific, toxic type were found in the livers of some of the guinea pigs and mice and in all of the rabbits and rats. Similar changes were also found in the renal tubules of the rats. Although microscopic abnormalities of this type are often found in the tissues of "normal" animals, those observed in certain of the control animals were not as severe as they were found to be in the corresponding species of animals that had been exposed to Aroclor 1254. The conclusion seems to be warranted, therefore, that prolonged respiratory exposure to Aroclor 1254 is capable of causing some injury to the tissues of susceptible animals, under conditions in which the atmospheric concentration of the material is of the order of 0.11 parts per million.

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Exposure to the Vapors

The method of volatilization of either of the Aroclors from a heated glass well was similar to that described in the earlier report (June 22, 1955), except that in these experiments the temperatures of the Aroclors in their respective wells were decreased and the rates of air-flow over the surface of the liquids were increased. In the case of Aroclor 1242, which was heated to 55° to 60°C, the air passed over the liquid at the rate of 600 liters per minute as measured by a venturi-meter attached to an inclined manometer. Aroclor 1254 was maintained between 115° and 125°C while 700 liters of air per minute passed over its surface before entering the chamber.

During a 7-hour period on each of 5 days per week, 3 groups of animals (2 experimental and 1 control) were confined simultaneously in separate 600 liter chambers as described earlier. Each group consisted originally of 1 cat, 6 guinea pigs, 10 mice, 4 rabbits and 10 rats. When an animal in any of these groups died, it was replaced soon thereafter.

The content of Aroclor in the air was determined daily by the turbidimetric method described in the previous report. In these experiments samples were collected by passing air at the rate of 1 liter per minute for 45 minutes through a Willson

combustion furnace and then through 2 midget bubblers in series, each containing 10 ml of 0.1 N sodium hydroxide.

Experimental Results

Aroclor 1242, Experiment No. 3. The pertinent data relating to the fate of the individual animals of this experiment are detailed in Table 1 and summarized in Table 4. One cat, 5 guinea pigs, 6 mice and 9 rats survived following their subjection for 7 hours on each of 150 days (over 214 days) to air bearing the vapor of Aroclor 1242 in the concentration of 1.9 micrograms per liter (0.18 ppm). Seven additional animals, introduced as substitutes for animals that had died during the experimental period, were alive at the conclusion of the final period of exposure; these are listed to show their species and, in parentheses, the number of periods of exposure to which each was subjected is given, as follows: 1 guinea pig (26), 2 mice (45, 135), 3 rabbits (8, 13 and 34), and 1 rat (107).

In the initial group, 10 fatalities occurred as follows: 1 guinea pig after 124 periods of exposure; 4 mice after 14, 14, 102 and 150 periods, respectively; 4 rabbits after 17, 116, 118 and 129 periods, respectively; and 1 rat after 43 periods. In addition, a substituted mouse died after 135 periods of

exposure, and 11 substituted rabbits died, for the most part, during the first 2 weeks after they had been introduced. Ten of the 15 rabbits died within a period of 3 weeks during a severe epidemic of pneumonia.

Except in the case of the rabbits, the incidence of mortality was no greater among the experimental animals than among the controls (Tables 3 and 4). Among the original group of controls 1 cat, 5 guinea pigs, 4 mice, 2 rabbits and 9 rats survived throughout the entire period of their confinement, for 7 hours on each of 150 days over 213 days in a chamber supplied with conditioned air. Three other animals, 1 rabbit and 2 mice, were killed after 34, 109 and 113 periods of confinement, respectively, in a chamber in which air containing no Aroclor was conditioned with respect to dust, humidity and temperature. Other control animals, introduced as substitutes for those that had died or been killed, survived during the indicated numbers of periods of confinement, respectively: 1 guinea pig, 55; 6 mice, 5, 5, 13, 37, 41 and 66; 2 rabbits, 115 and 119; and 1 rat, 115. Seven fatalities occurred among the original group as follows: 1 guinea pig after 94 periods of confinement; 4 mice after 84, 112, 137 and 141 periods, respectively; 1 rabbit after 30 periods, and 1 rat after 29. Two mice used as substitutes died after 4 and 33 periods of confinement, respectively.

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In summary, in each of the original (experimental and control) groups, 1 guinea pig, 4 mice and 1 rat died.

No specific signs of intoxication were observed in the members of either the experimental or control groups.

The weights, observed at weekly intervals, of the individual animals (cats, guinea pigs, mice, rabbits and rats) exposed to Aroclor 1242 (Experiment No. 3) are shown in Figures 1 to 3, 5 to 7 and 9 to 14. The average weight of the original groups and the greatest and least weights attained by individuals within these groups of guinea pigs, mice and rats that survived are plotted in Figures 4, 8 and 14, respectively. Comparable data for the controls are to be found in Figures 30 to 44. The average changes in weight of the various survivors of the original groups (experimental and control) are shown in Table 5. The application of the widely used "t" test indicates that the growth of the animals exposed to Aroclor 1242 did not differ significantly from that of the control group.

All of the animals were examined post-mortem, and the viscera of most of them were examined microscopically. Among the exposed group, 1 guinea pig, 5 mice and 15 rabbits that died, and 1 rat that was killed when moribund, were victims of incidental infectious disease. The cat, guinea pigs, mice and rats that survived had normal viscera. The 3 rabbits used as

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replacements survived. Two of them were found to have hepatic lesions of coccidiosis and the other had no gross microscopic abnormalities.

The control animals that died were found to have pneumonia. Of those that survived, 1 rat, 2 guinea pigs and 1 mouse had focal or diffuse cytoplasmic vacuolation of the hepatic cells, the etiology of which was not apparent. The remaining animals that survived had essentially normal viscera.

Aroclor 1254, Experiment No. 2. The pertinent data relating to the changes in weight and fate of the individual animals subjected to prolonged intermittent exposure to the vapor of Aroclor 1254 are shown in Table 2 and summarized in Table 4. One cat, 4 guinea pigs, 6 mice, 4 rabbits and 9 rats from the original group survived following their exposure for 7 hours on each of 150 days over a period of 213 days to air containing Aroclor 1254 in the concentration of 1.5 micrograms per liter (0.11 ppm). One guinea pig and 1 rat from the original group were killed after 30 and 149 periods of exposure, respectively. Seven animals introduced into the group later survived. They are listed by species and periods of exposure, in parentheses, as follows: 2 guinea pigs (49 and 120); 4 mice (49, 66, 115 and 135); 1 rat (139). Among the original group only 4 mice and 1 guinea pig died after 12 to 101 periods of exposure, and

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a mouse, which was a replacement, died after 18 periods of exposure, the incidence of fatalities among them being slightly less than that encountered among the controls.

No general or specific signs of intoxication were noted among the experimental animals during or after their exposure.

The weights, at weekly intervals, of the individual animals (i.e., cat, guinea pigs, mice, rabbits and rats) exposed to Aroclor 1254 are presented in Figures 15 to 17, 19 to 21, 23 and 24, and 26 to 28. The average weight of the original groups, and the greatest and least weights attained by individuals within these groups of guinea pigs, mice, rabbits and rats that survived are presented graphically in Figures 18, 22, 25 and 29, respectively. The rates of growth of the experimental groups did not differ significantly from those of the control group (Table 5).

All of the animals were examined post-mortem, and the viscera of most of them were examined microscopically. The guinea pig that died exhibited chronic pyelonephritis, pulmonary hyperemia and edema, and degenerative lesions in the brain and liver. The visceral lesions were related, no doubt, to the renal infection. The deaths of the 4 mice were attributable to acute bronchitis and pneumonia. The viscera of the cat that survived were normal. Of 7 guinea pigs that were living when the experiment was terminated, 3 had normal viscera and four had slight

alterations of hepatic cells characterized by cytoplasmic vacuolation. Ten mice survived and of these 6 had normal viscera and 4 had slight degenerative changes in the liver. Four rabbits killed 1 to 15 days after the last period of exposure had diffuse hepatic degeneration. The character of the lesions varied from cloudy, hyaline or hydropic degeneration and included varying degrees of fatty metamorphosis. The other viscera of these animals were normal. All of the rats were examined and found to have degenerative lesions of the liver of slight to moderate degree. The lesions of greatest severity were found in the rat that was killed and examined on the first day after the last period of exposure. Two rats had chronic pyelonephritis, and the remainder had slight degeneration of the renal tubules.

Discussion

The preceding report (dated June 22, 1955) indicated that no harm resulted to animals that inhaled Aroclor 1242, intermittently, over periods not exceeding 4 months when the concentration in the atmosphere approached that of saturation. The present report demonstrates, in terms of rates of growth, incidence of mortality and non-occurrence of pathologic changes,

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that no injurious effects resulted from more prolonged, intermittent exposure (more than 6 months) to the concentration of 0.18 ppm in the atmosphere (1.9 micrograms per liter). This concentration is nearly twice that (1 microgram per liter) recommended by the American Conference of Governmental Hygienists (Arch. Ind. Hyg. Occup. Med., 9:530, 1954) for a chlorinated diphenyl of unstated chlorine content.

The exposure of animals to Aroclor 1254 failed to induce harmful effects in the form of retardation of growth, or of mortality, but histopathologic evidence of apparently reversible hepatic cellular injury was found in the animals. These findings cannot certainly be attributed to the effects of Aroclor 1254 because of the appreciable incidence of pneumonia among both experimental and control animals. When these nonspecific toxic changes in the viscera of the animals were associated with pneumonia, they were readily explained thereby, but they were also found in animals that had been exposed to the vapor of Aroclor 1254 and were free of pneumonia. That these may have represented toxic effects of exposure to Aroclor 1254 finds support in the fact that the livers of the exposed rats (report of June 22, 1955) were significantly heavier in relation to their body weight, than were those of control rats. It would appear that this material, which is reported to contain 54

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per cent of chlorine, is somewhat more toxic than is Aroclor 1242, which contains only 42 per cent of chlorine. O.K. Drinker proposed (J. Ind. Hyg. Toxicol., 21:155, 1939) as a threshold concentration for a sample of a chlorinated diphenyl that contained 50 to 55 per cent of chlorine, the value of 0.5 microgram per liter, which is about one-third of the concentration of Aroclor 1254 to which the animals were exposed in this experiment.

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Project No. 47.

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Table 1
The Mortality, Length of Survival, and Changes in Weight of
Animals Exposed to the Vapor of Aroclor 1242 in Air
(Experiment No. 3)

Concentration 7/1 ppm	Duration of Exposure (hours)	Identification Number	Initial Weight (kg)	Change in Weight Expressed as Percentage of Initial Weight		Fate	Length of Sur- vival After Last Period of Exposure Unless Otherwise Stated
				During Period of Exposure	After Period of Exposure		
1.9	0.18	150 x 7.0	1.730	+ 115.0	- 2.6	Survived	Killed 8 days
		150 x 7.0	434	+ 22.8	-	Survived	Killed 1 day
		124 x 7.0	476	+ 34.2	-	Died	Killed 1 day
		150 x 7.0	462	+ 36.8	+ 0.8	Survived	Killed 8 days
		150 x 7.0	583	+ 66.0	+ 7.0	Survived	Killed 8 days
		150 x 7.0	570	+ 36.7	+ 3.8	Survived	Killed 15 days
		150 x 7.0	520	+ 53.1	+ 2.7	Survived	Killed 15 days
		26 x 7.0	562	+ 1.1	+ 4.1	Survived	Killed 8 days
		150 x 7.0	22	+ 4.5	-	Survived	Killed 1 day
		150 x 7.0	24	+ 16.7	+ 4.2	Survived	Killed 8 days
		14 x 7.0	27	- 11.1	-	Died	Killed 1 day
		102 x 7.0	25	-	-	Died	Killed 1 day
		150 x 7.0	24	+ 25.0	-	Survived	Killed 8 days
		150 x 7.0	21	+ 52.4	+ 9.5	Survived	Killed 8 days
		150 x 7.0	28	+ 17.8	+ 28.6	Survived	Killed 8 days
		150 x 7.0	24	+ 12.5	+ 4.17	Survived	Killed 8 days
		150 x 7.0	23	- 13.0	+ 8.7	Died	Killed 12 days
		14 x 7.0	25	- 52.0	-	Died	Killed 1 day

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Table 1 (Page 2)

Concentration γ/l.	Duration of Exposure (hours)	Identification Number	Initial Weight (kg)	Change in Weight Expressed as Percentage of Initial Weight		Fate	Length of Sur- vival After Last Period of Exposure Unless Otherwise Stated
				During Period of Exposure	After Period of Exposure		
1.9	135 x 7.0	Mouse B-600	23	+ 21.7	+ 4.35	Survived	Killed 15 days
	135 x 7.0	Mouse B-601	23	- 4.35	- 21.7	Died	13 days
	45 x 7.0	Mouse B-921	27	+ 22.2	-	Survived	Killed 15 days
	129 x 7.0	Rabbit H-1061	3058	+ 18.8	-	Died	1 day
	118 x 7.0	Rabbit H-1062	2688	+ 19.3	-	Died	1 day
	17 x 7.0	Rabbit H-1063	2150	- 11.6	-	Died	1 day
	116 x 7.0	Rabbit H-1064	3022	+ 48.7	- 5.7	Died	3 days
	31 x 7.0	Rabbit J-84	2885	+ 1.0	-	Died	1 day
	79 x 7.0	Rabbit J-324	2431	+ 38.5	-	Died	1 day
	34 x 7.0	Rabbit J-760	2833	+ 10.8	-	Survived	Killed 1 day
	12 x 7.0	Rabbit J-784	2566	- 7.9	-	Died	1 day
	4 x 7.0	Rabbit J-822	3729	- 3.3	-	Died	2 hours
	3 x 7.0	Rabbit J-823	3980	- 6.0	-	Died	During 3rd run
	7 x 7.0	Rabbit J-828	2887	- 11.9	-	Died	0.5 day
	5 x 7.0	Rabbit J-861	3415	- 17.2	-	Died	During 5th run
	7 x 7.0	Rabbit J-862	3343	+ 0.6	- 2.6	Survived	2 days
	13 x 7.0	Rabbit J-863	3425	+ 2.9	+ 5.4	Died	Killed 8 days
	2 x 7.0	Rabbit J-864	3851	- 5.9	- 4.9	Died	2 days
	2 x 7.0	Rabbit J-885	2650	- 14.3	-	Died	0.5 day
	8 x 7.0	Rabbit J-886	2390	+ 6.2	+ 14.1	Survived	Killed 15 days
	6 x 7.0	Rabbit J-887	3592	- 18.7	- 4.7	Died	3 days
	150 x 7.0	Rat L-992	240	+ 28.8	-	Survived	Killed 1 day
	150 x 7.0	Rat L-993	197	+ 33.0	-	Survived	Killed 8 days

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Table 1 (Page 3)

Concentration mg/l.	Duration of Exposure (hours)	Identification Number	Initial Weight (kg)	Change in Weight Expressed as Percentage of Initial Weight		Fate	Length of Sur- vival After Last Period of Exposure Unless Otherwise Stated
				During Period of Exposure	After Period of Exposure		
1.9	150 ± 7.0	Rat L-994	215	+ 42.8	+ 4.2	Survived	Killed 8 days
	150 ± 7.0	Rat L-995	232	+ 25.0	+ 3.0	Survived	Killed 8 days
	150 ± 7.0	Rat L-996	213	+ 26.8	- 1.9	Survived	Killed 8 days
	150 ± 7.0	Rat L-997	196	+ 34.2	- 0.5	Survived	Killed 8 days
	150 ± 7.0	Rat L-998	225	+ 58.2	+ 4.0	Survived	Killed 15 days
	150 ± 7.0	Rat L-999	189	+ 69.3	- 0.5	Survived	Killed 15 days
	150 ± 7.0	Rat L-1000	216	+ 41.2	- 0.46	Survived	Killed 15 days
	43 ± 7.0	Rat M-1	202	- 2.5	-	Killed	Moribund, 1 day
	107 ± 7.0	Rat M-445	174	+ 29.9	+ 1.7	Survived	Killed 15 days

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Table 2
The Mortality, Length of Survival, and Changes in Weight of
Animals Exposed to the Vapor of Aroclor 1254 in Air

Concentration		Duration of Exposure (hours)	Identification Number	Initial Weight (kg)	Change in Weight Expressed as Percentage of Initial Weight		Fate	Length of Survival After Last Period of Exposure Unless Otherwise Stated
g/l	ppm				During Period of Exposure	After Period of Exposure		
1.5	0.11	150 x 7.0	Cat A-500	2858	+ 15.2	- 2.9	Survived	Killed 8 days
		30 x 7.0	Guinea Pig B-605	478	+ 7.9	-	Survived	Killed 1 day
		150 x 7.0	Guinea Pig B-606	515	+ 21.0	+ 2.1	Survived	Killed 8 days
		101 x 7.0	Guinea Pig B-607	544	+ 34.9	-	Died	2 hours
		150 x 7.0	Guinea Pig B-608	483	+ 67.1	+ 7.7	Survived	Killed 8 days
		150 x 7.0	Guinea Pig B-609	475	+ 26.3	+ 6.7	Survived	Killed 15 days
		150 x 7.0	Guinea Pig B-610	476	+ 15.5	+ 6.9	Survived	Killed 15 days
		120 x 7.0	Guinea Pig B-672	541	+ 23.8	-	Survived	Killed 1 day
		49 x 7.0	Guinea Pig B-799	550	+ 8.2	- 0.9	Survived	Killed 8 days
		150 x 7.0	Mouse B-577	25	- 12.0	-	Survived	Killed 1 day
		150 x 7.0	Mouse B-578	21	+ 28.6	+ 4.8	Survived	Killed 9 days
		150 x 7.0	Mouse B-579	25	+ 24.0	+ 4.0	Survived	Killed 9 days
		150 x 7.0	Mouse B-580	22	+ 31.8	-	Survived	Killed 9 days
		101 x 7.0	Mouse B-581	30	- 20.0	-	Died	2 hours
		150 x 7.0	Mouse B-582	30	+ 3.3	-	Survived	Killed 9 days
		14 x 7.0	Mouse B-583	25	+ 4.0	-	Died	0.5 day
		12 x 7.0	Mouse B-584	29	- 13.8	-	Died	0.5 day
		150 x 7.0	Mouse B-585	23	+ 26.0	- 4.3	Survived	Killed 8 days

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Table 2 (Page 2)

Concentration ppm	Duration of Exposure (hours)	Identification Number	Initial Weight (kg)	Change in Weight Expressed as Percentage of Initial Weight		Fate	Length of Sur- vival After Last Period of Exposure Unless Otherwise Stated
				During Period of Exposure	After Period of Exposure		
1.5	0.11						
	83 x 7.0	Mouse B-586	28	+ 36.4	+ 4.5	Died	1 day
	135 x 7.0	Mouse B-602	22	+ 26.1	- 21.7	Survived	Killed 15 days
	18 x 7.0	Mouse B-603	23			Died	1 day
	115 x 7.0	Mouse B-673	22	+ 22.7	- 4.5	Survived	Killed 15 days
	66 x 7.0	Mouse B-863	18	+ 72.2	- 16.7	Survived	Killed 15 days
	49 x 7.0	Mouse B-919	19	+ 15.8	+ 10.5	Survived	Killed 15 days
	150 x 7.0	Rabbit H-1065	2740	+ 40.1	-	Survived	Killed 1 day
	150 x 7.0	Rabbit H-1066	3457	+ 53.5	+ 5.1	Survived	Killed 8 days
	150 x 7.0	Rabbit H-1067	3000	+ 43.0	+ 1.5	Survived	Killed 8 days
	150 x 7.0	Rabbit H-1068	2633	+ 41.1	+ 3.8	Survived	Killed 15 days
	150 x 7.0	Rat M-2	247	+ 22.7	-	Survived	Killed 1 day
	150 x 7.0	Rat M-3	214	+ 32.7	+ 2.3	Survived	Killed 8 days
	150 x 7.0	Rat M-4	228	+ 37.3	+ 1.7	Survived	Killed 8 days
	150 x 7.0	Rat M-5	202	+ 33.2	- 5.0	Survived	Killed 8 days
	150 x 7.0	Rat M-6	208	+ 10.6	- 9.6	Survived	Killed 8 days

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Table 2 (Page 3)

Concentration γ/l	ppm	Duration of Exposure (hours)	Identification Number	Initial Weight (kg)	Change in Weight Expressed as Percentage of Initial Weight			Fate	Length of Sur- vival After Last Period of Exposure Unless Otherwise Stated
					During Period of Exposure	After Period of Exposure			
1.5	0.11	150 ± 7.0	Rat M-7	206	+ 15.5	+ 11.2	Survived	Killed 8 days	
		149 ± 7.0	Rat M-8	226	+ 49.6	- 7.5	Survived	Killed 15 days	
		150 ± 7.0	Rat M-9	222	+ 37.8	+ 1.8	Survived	Killed 15 days	
		150 ± 7.0	Rat M-10	225	+ 29.8	- 0.9	Survived	Killed 15 days	
		150 ± 7.0	Rat M-11	243	+ 18.1	- 0.4	Survived	Killed 15 days	
		139 ± 7.0(1)	Rat M-140	226	+ 11.9	- 1.3	Survived	Killed 15 days	

(1) An eleventh rat was added to this group after the 11th period of exposure.

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Table 3
The Mortality, Length of Survival, and Changes in Weight
of Animals Exposed to Conditioned Air

Duration of Exposure (hours)	Identification Number	Initial Weight (kg)	Change in Weight Expressed as Percentage of Initial Weight		Rate	Length of Survival After Last Period of Exposure Unless Otherwise Stated
			During Period of Exposure	After Period of Exposure		
150 x 7.0	Oat A-510	3329	- 7.8	- 2.1	Survived	Killed 8 days
94 x 7.0	Guinea Pig B-611	434	- 34.3	- 3.0	Died	1 day
150 x 7.0	Guinea Pig B-612	516	+ 78.7	+ 4.3	Survived	Killed 9 days
150 x 7.0	Guinea Pig B-613	542	+ 24.4	-	Survived	Killed 1 day
150 x 7.0	Guinea Pig B-614	477	+ 52.2	+ 5.5	Survived	Killed 9 days
150 x 7.0	Guinea Pig B-615	453	+ 42.4	+ 1.3	Survived	Killed 15 days
150 x 7.0	Guinea Pig B-616	514	+ 75.7	+ 1.2	Survived	Killed 15 days
55 x 7.0	Guinea Pig B-798	640	- 8.9	+ 0.2	Survived	Killed 9 days
141 x 7.0	Mouse B-587	30	- 46.7	-	Died	1 day
150 x 7.0	Mouse B-588	27	+ 11.1	-	Survived	Killed 1 day
150 x 7.0	Mouse B-589	26	+ 30.8	- 3.8	Survived	Killed 9 days
84 x 7.0	Mouse B-590	27	-	-	Died	1 day
113 x 7.0	Mouse B-591	25	+ 4.0	- 14.0	Survived	Killed acutely, 4 days
137 x 7.0	Mouse B-592	27	- 7.4	- 11.1	Died	3 days
112 x 7.0	Mouse B-593	24	- 37.5	-	Died	5 hours

(Experiment No. 2)

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Table 3 (Page 2)

Duration of Exposure (hours)	Identification Number	Initial Weight (kg)	Change in Weight Expressed as Percentage of Initial Weight		Fate	Length of Survival After Last Period of Exposure Unless Otherwise Stated
			During Period of Exposure	After Period of Exposure		
109 x 7.0	Mouse B-594	24	- 16.6	-	Survived	Killed accidentally, 1 hour
150 x 7.0	Mouse B-595	25	+ 20.0	- 4.0	Survived	Killed 9 days
150 x 7.0	Mouse B-596	27	+ 29.6	+ 3.7	Survived	Killed 9 days
66 x 7.0	Mouse B-864	20	+ 35.0	-	Survived	Killed 9 days
41 x 7.0	Mouse B-933	21	+ 14.3	- 4.8	Survived	Killed 9 days
33 x 7.0	Mouse B-935	22	-	-	Died	1 day
37 x 7.0	Mouse B-936	19	+ 21.05	+ 5.3	Survived	Killed 15 days
13 x 7.0	Mouse B-967	24	4.2	-	Survived	Killed 15 days
4 x 7.0	Mouse B-968	22	-	-	Died	0.5 day
5 x 7.0	Mouse B-969	22	+ 9.1	+ 13.6	Survived	Killed 15 days
5 x 7.0	Mouse B-970	22	+ 4.5	-	Survived	Killed 15 days
150 x 7.0	Rabbit H-1069	2728	+ 31.0	-	Survived	Killed 1 day
150 x 7.0	Rabbit H-1070	2907	+ 27.1	+ 2.7	Survived	Killed 8 days
30 x 7.0	Rabbit H-1071	3132	+ 16.5	- 2.6	Died	2 days
34 x 7.0	Rabbit H-1072	2800	+ 8.2	-	Survived	Killed 1 day
119 x 7.0	Rabbit J-183	2661	+ 66.5	+ 7.9	Survived	Killed 8 days
115 x 7.0	Rabbit J-202	2193	+ 84.2	+ 9.3	Survived	Killed 15 days

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Table 3 (Page 3)

Duration of Exposure (hours)	Identification Number	Initial Weight (kg)	Change in Weight Expressed as Percentage of Initial Weight		Fate	Length of Survival After Last Period of Exposure Unless Otherwise Stated
			During Period of Exposure	After Period of Exposure		
150 x 7.0	Rat M-12	219	+ 32.4	-	Survived	Killed 1 day
150 x 7.0	Rat M-13	206	+ 44.2	+ 1.4	Survived	Killed 8 days
150 x 7.0	Rat M-14	207	+ 40.1	+ 2.9	Survived	Killed 8 days
150 x 7.0	Rat M-15	263	+ 30.0	+ 1.5	Survived	Killed 8 days
150 x 7.0	Rat M-16	208	+ 30.3	+ 2.9	Survived	Killed 8 days
150 x 7.0	Rat M-17	213	+ 34.3	+ 0.9	Survived	Killed 8 days
150 x 7.0	Rat M-18	212	+ 26.9	+ 0.5	Survived	Killed 15 days
29 x 7.0	Rat M-19	212	- 13.2	- 10.8	Died	5 days
150 x 7.0	Rat M-20	214	+ 36.4	-	Survived	Killed 15 days
150 x 7.0	Rat M-21	223	+ 28.7	+ 1.3	Survived	Killed 15 days
115 x 7.0	Rat M-351	186	+ 58.6	+ 1.6	Survived	Killed 15 days

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Table 4

Summary of the Data on the Mortality Among Experimental* and Control Animals

Species of Animal	Original Group		Replacements	
	Number that Survived (Number of Periods)	Number that Died (Number of Periods)	Number that Survived (Number of Periods)	Number that Died (Number of Periods)
Experiment No. 3 - Aroclor 1242				
Cat	1(150)	-	-	-
Guinea Pigs	5(150)	1(124)	1(26)	-
Mice	6(150)	4(14, 14, 102, 150)	2(45, 135)	1(135)
Rabbits	0	4(17, 116, 118, 129)	3(8, 13, 34)	11(2, 2, 3, 4, 5, 6, 7, 7, 12, 31, 79)
Rats	9(150)	1(43)	1(107)	-
Experiment No. 2 - Aroclor 1254				
Cat	1(150)	-	-	-
Guinea Pigs	1(130); 4(150)	1(101)	2(49, 120)	-
Mice	6(150)	4(12, 14, 83, 101)	4(49, 66, 115, 135)	1(18)
Rabbits	4(150)	-	-	-
Rats	1(149); 9(150)	-	1(139)	-
Controls - Experiment No. 2				
Cat	1(150)	-	-	-
Guinea Pigs	5(150)	1(94)	1(55)	-
Mice	2(109, 113); 4(150)	4(84, 112, 137, 141)	6(5, 5, 13, 37, 41, 66)	2(4, 33)
Rabbits	1(34); 2(150)	1(30)	2(115, 119)	-
Rats	9(150)	1(29)	1(115)	-

* Experimental animals were subjected to exposure for 7 hours per day on 150 days; controls were confined correspondingly in uncontaminated atmosphere.

NEV 016681

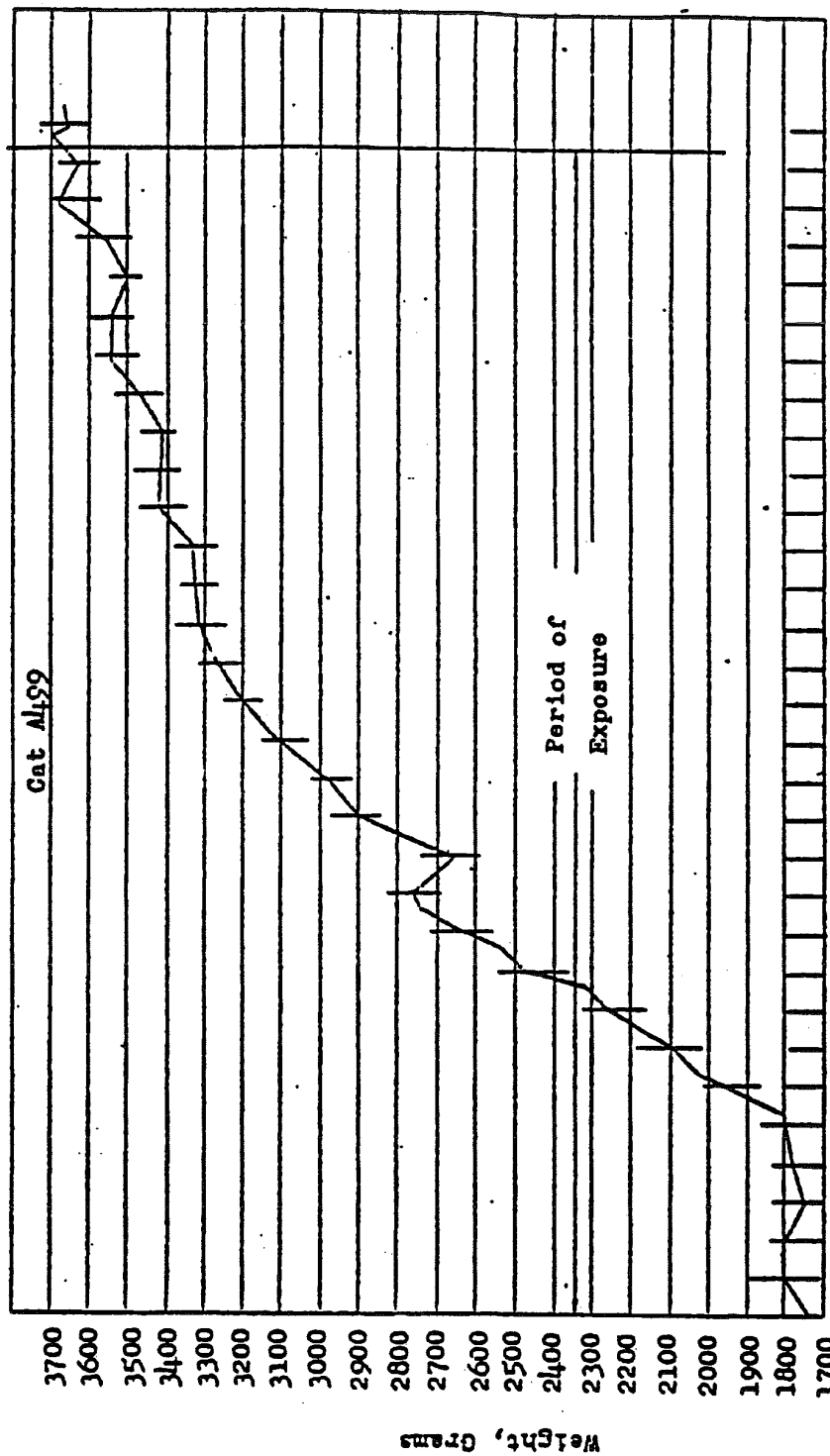
Table 5

The Average Changes in Weight of the Survivors
Among the Original Groups

Species of Animal	Number of Animals	Average Initial Weight (kg)	Average Change in Weight Expressed as Percentage of Initial Weight	P
Experiment No. 3 - Aroclor 1242				
Cat	1	1.730	+ 115.0	
Guinea Pigs	5	0.514	+ 43.1	>0.05
Mice	6	0.0238	+ 21.5	>0.05
Rats	9	0.214	+ 39.9	>0.05
Experiment No. 2 - Aroclor 1254				
Cat	1	2.858	+ 15.2	
Guinea Pigs	4	0.487	+ 32.5	>0.05
Mice	6	0.0243	+ 17.0	>0.05
Rabbits	4	2.958	+ 44.4	>0.05
Rats	10	0.222	+ 28.7	>0.05
Controls - Experiment No. 2				
Cat	1	3.329	- 7.8	
Guinea Pigs	5	0.500	+ 54.7	-
Mice	6	0.0257	+ 13.2	-
Rabbits	4 (1)	2.622	+ 52.2	-
Rats	9	0.218	+ 33.7	-

(1) Includes 2 early replacements.

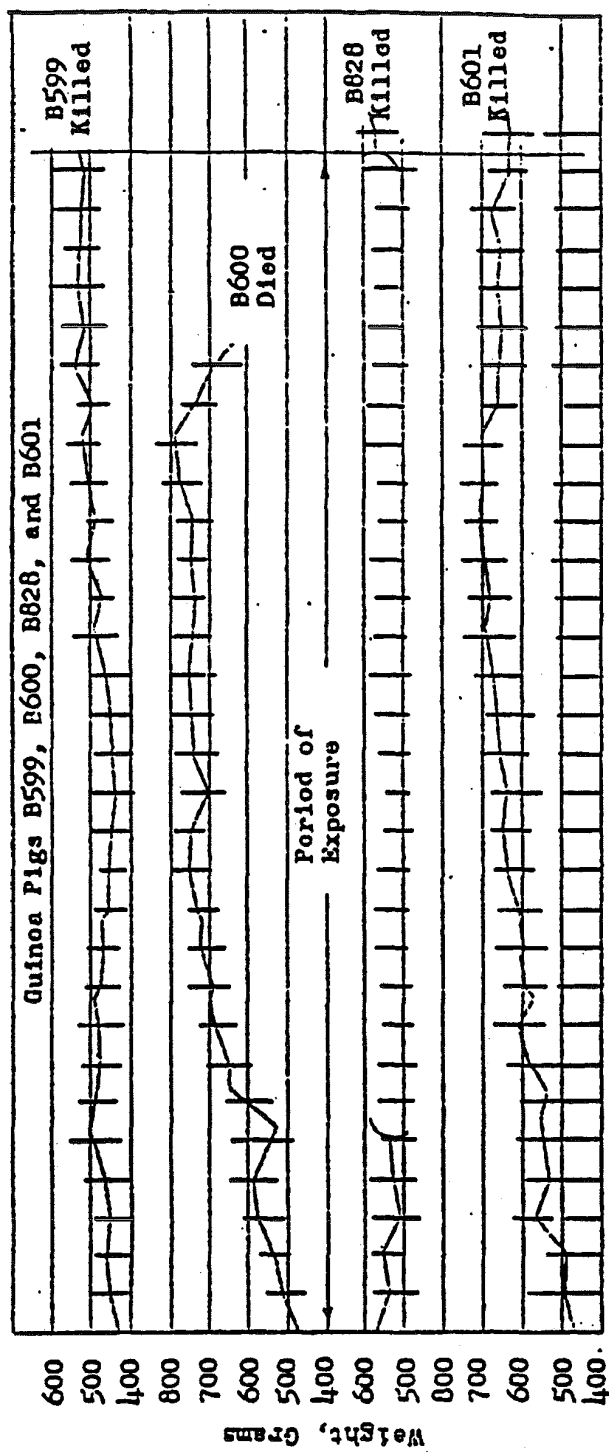
NEV 016682



Experiment No. 3
1.9 Micrograms of Aroclor 1242/liter

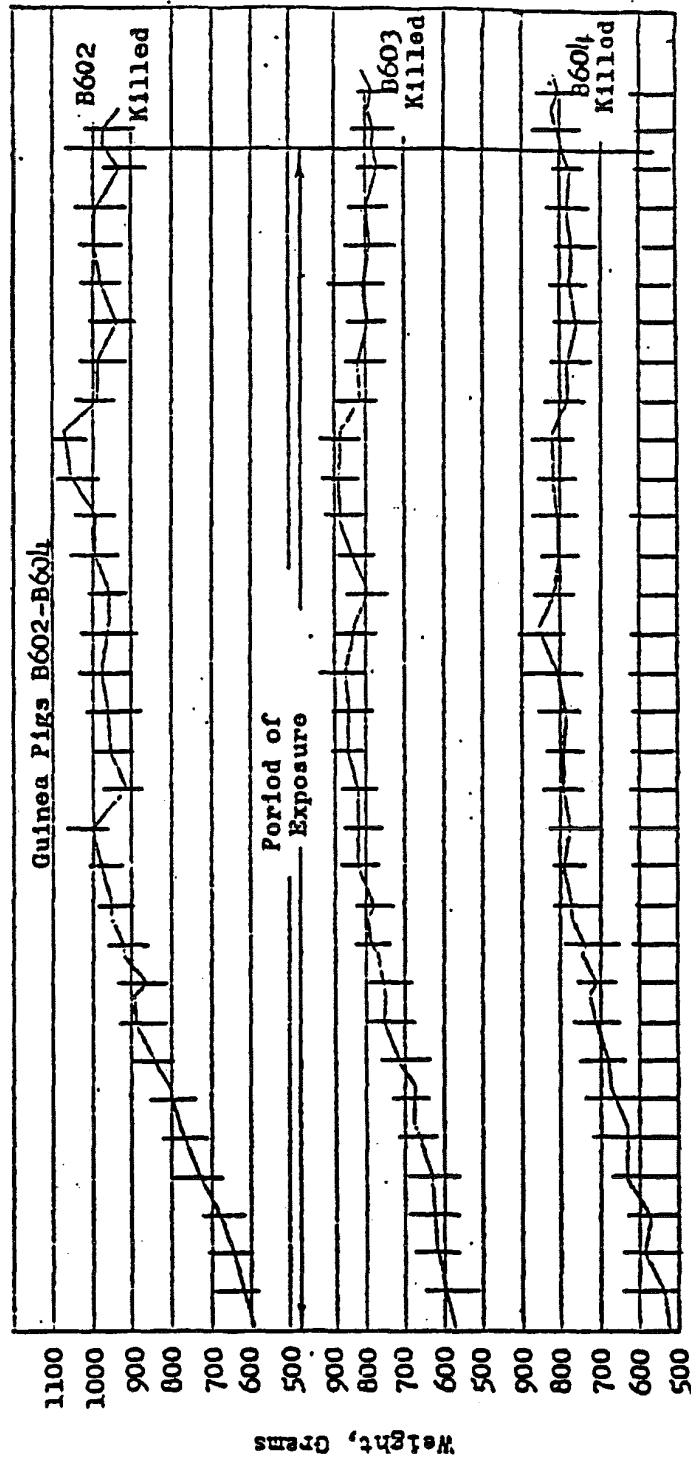
Figure 1

NEV 016683



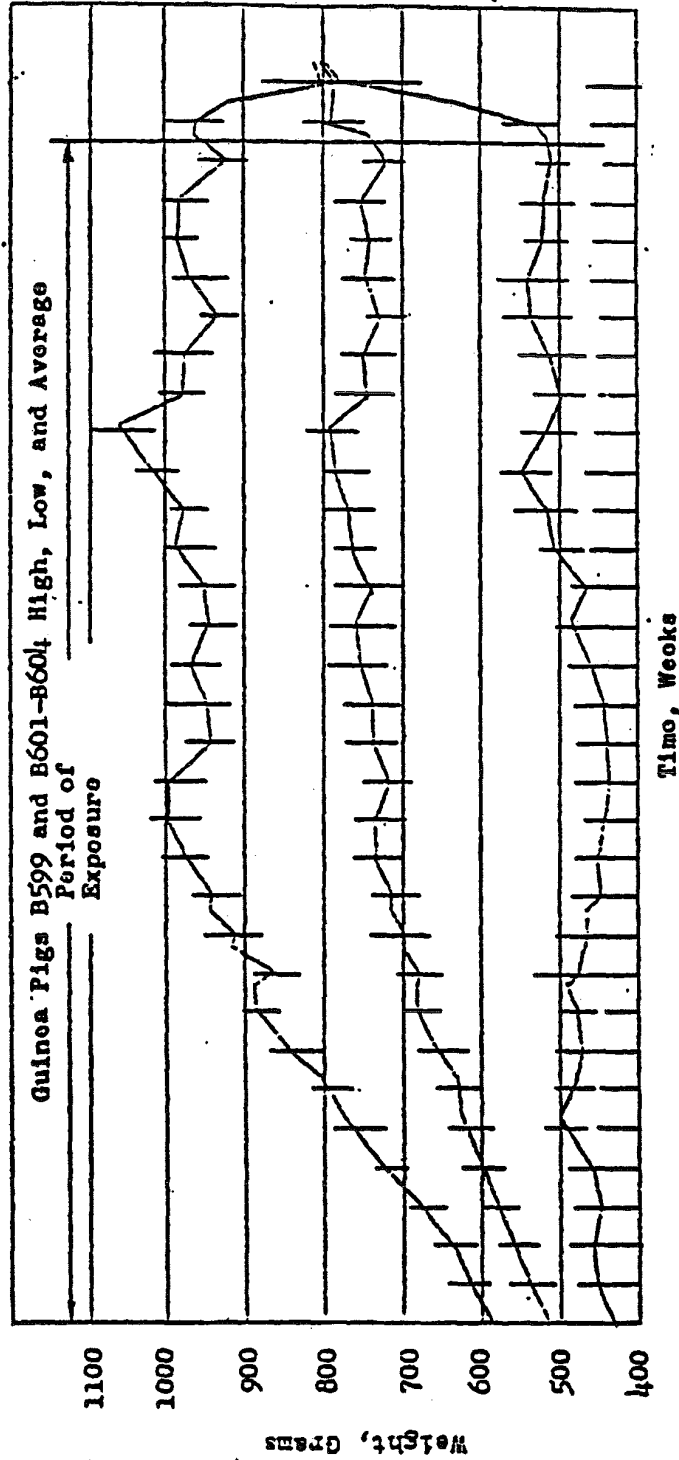
Experiment No. 3
1.9 Micrograms of Aroclor 1242/liter

NEV 016684



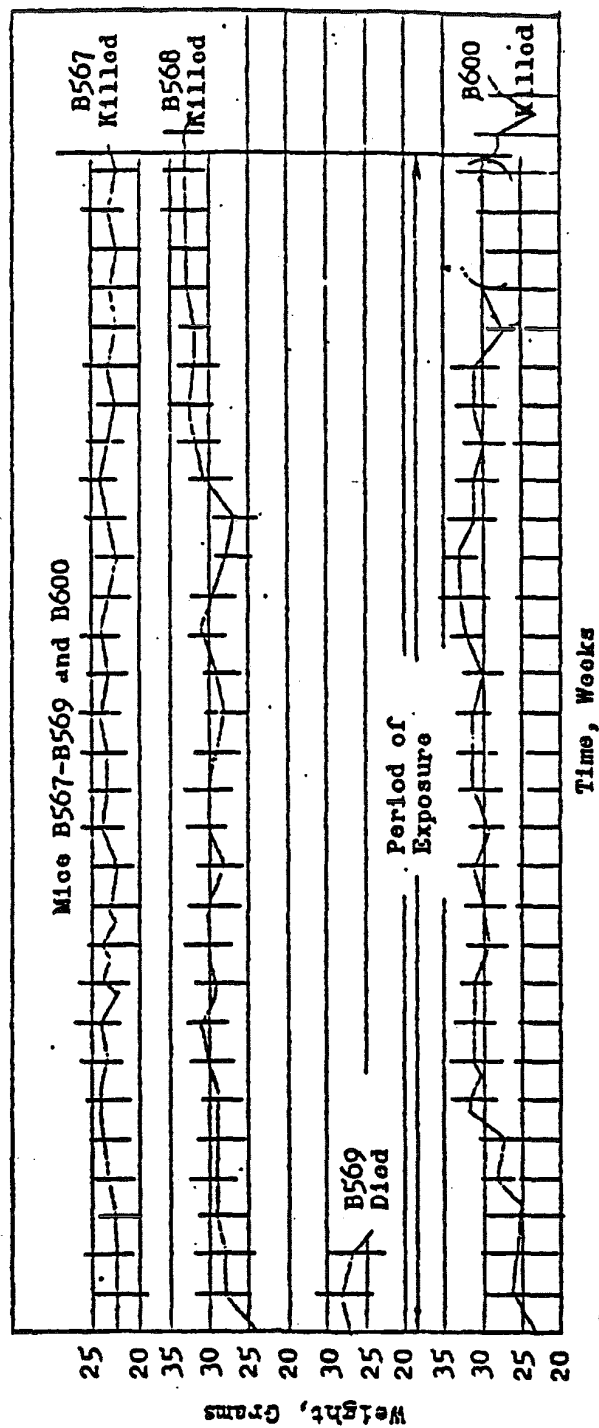
Experiment No. 3
1.9 Micrograms of Aroclor 1242/liter
Figure 3.

NEV 016685



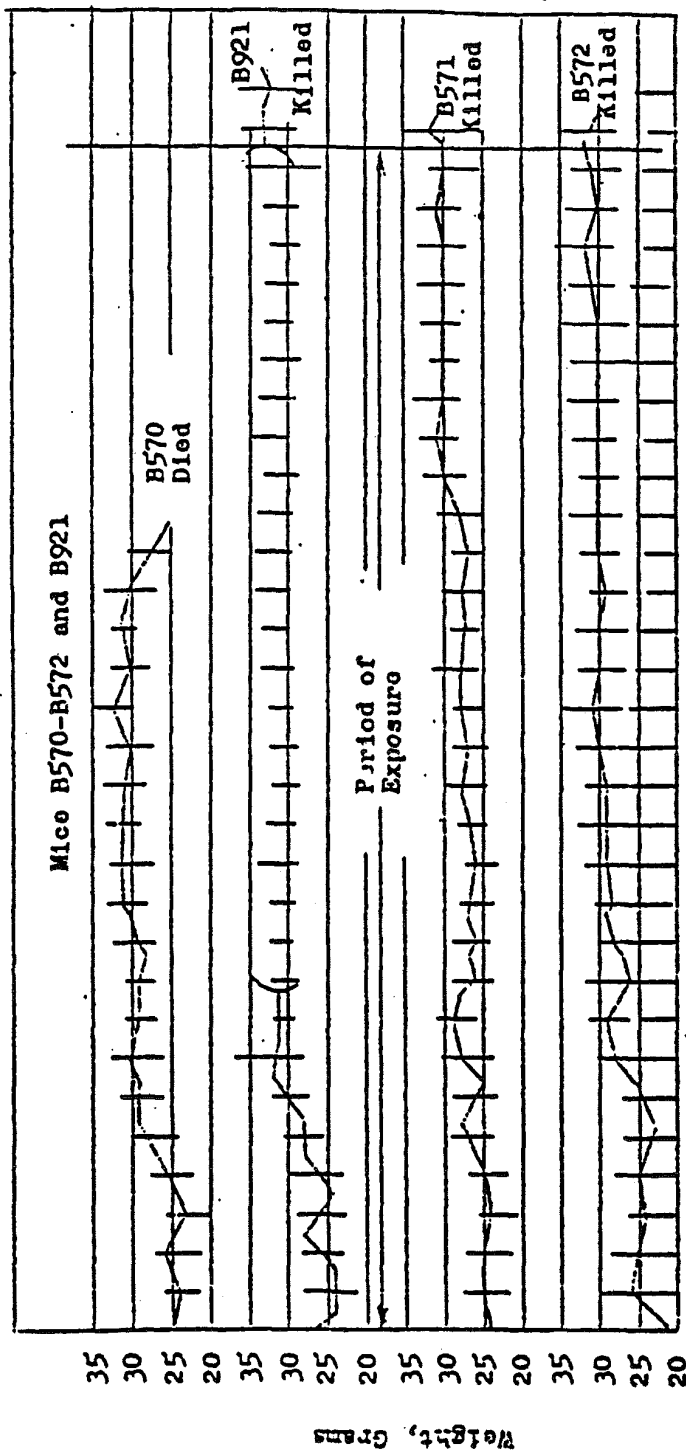
Experiment No. 3
1.9 Micrograms of aerosol 1242/11ter
Figure 4

NEV 016686



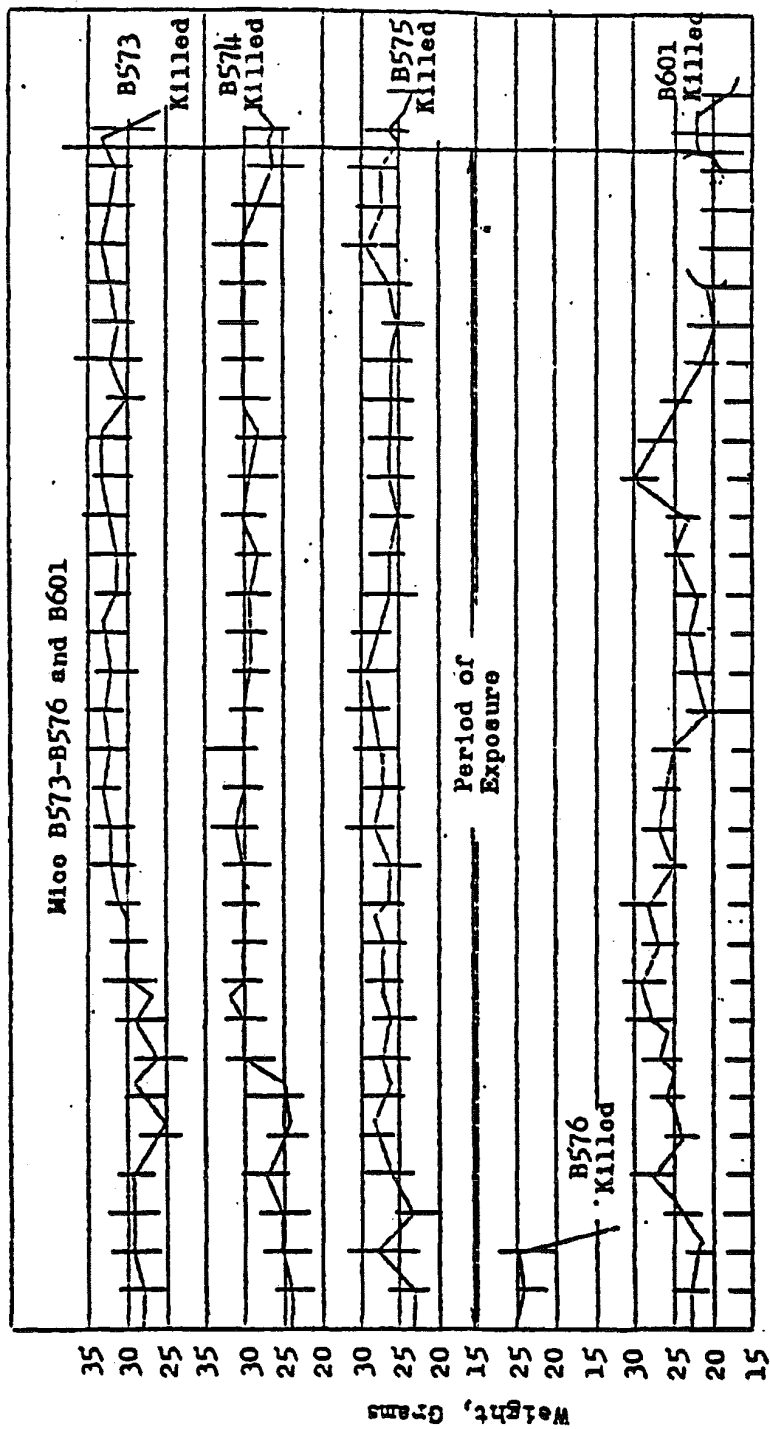
Experiment No. 3
1.9 Micrograms of Aroclor 1242/liter
Figure 5

NEV 016687



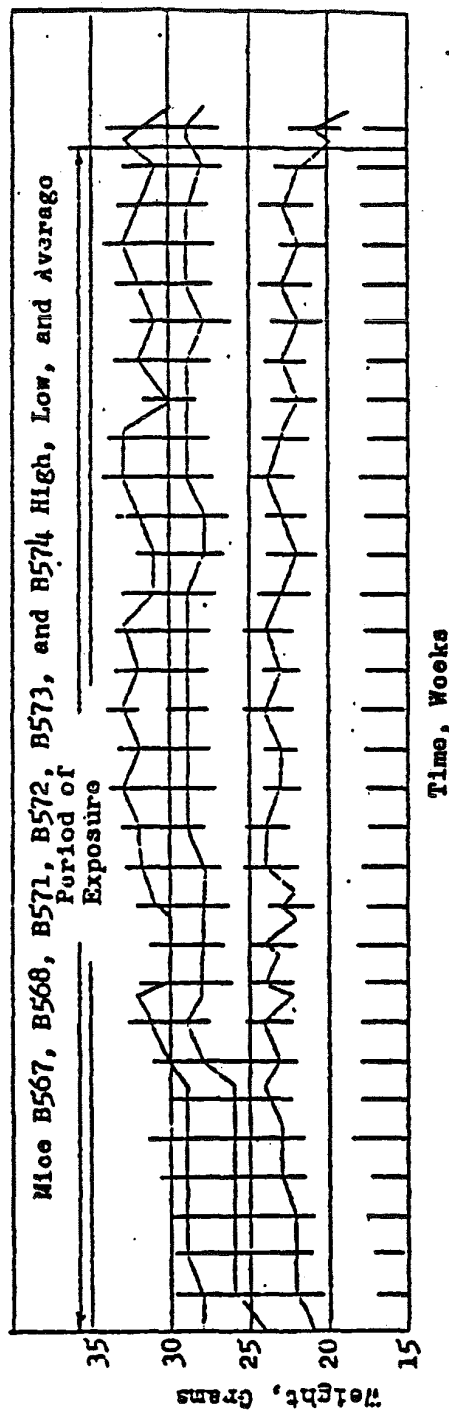
Experiment No. 3
 1.9 Micrograms of Aroclor 1242/liter
 Figure 6

NEV 016688



Experiment No. 3
1.9 Micrograms of Aroclor 1242/liter
Figure 7

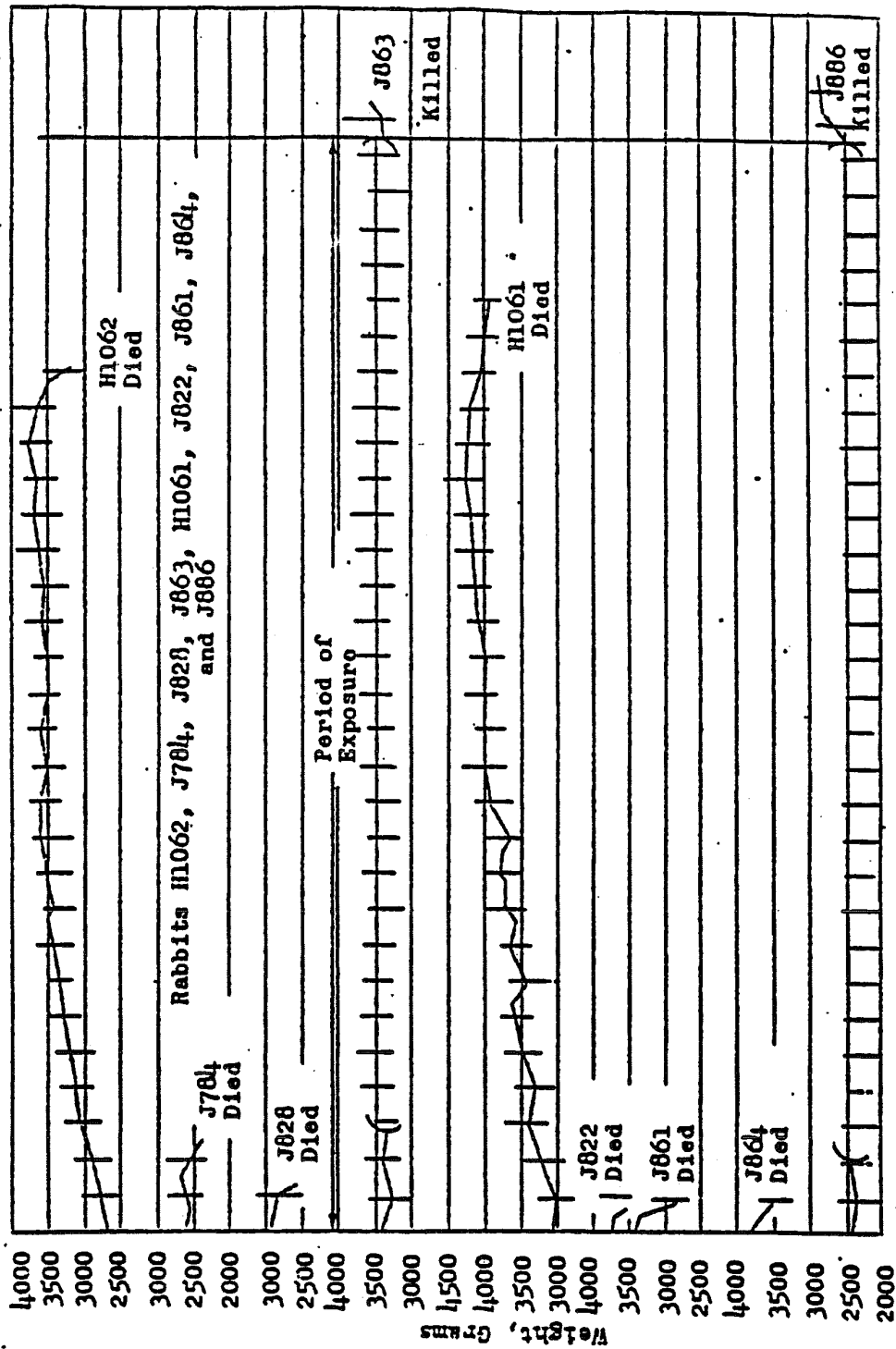
NEV 016689



Time, Weeks

Mice B567, B568, B571, B572, B573, and B574 High, Low, and Average
 Period of Exposure
 Experiment No. 3
 1.9 Micrograms of Aroclor 1242/1liter
 Figure 8

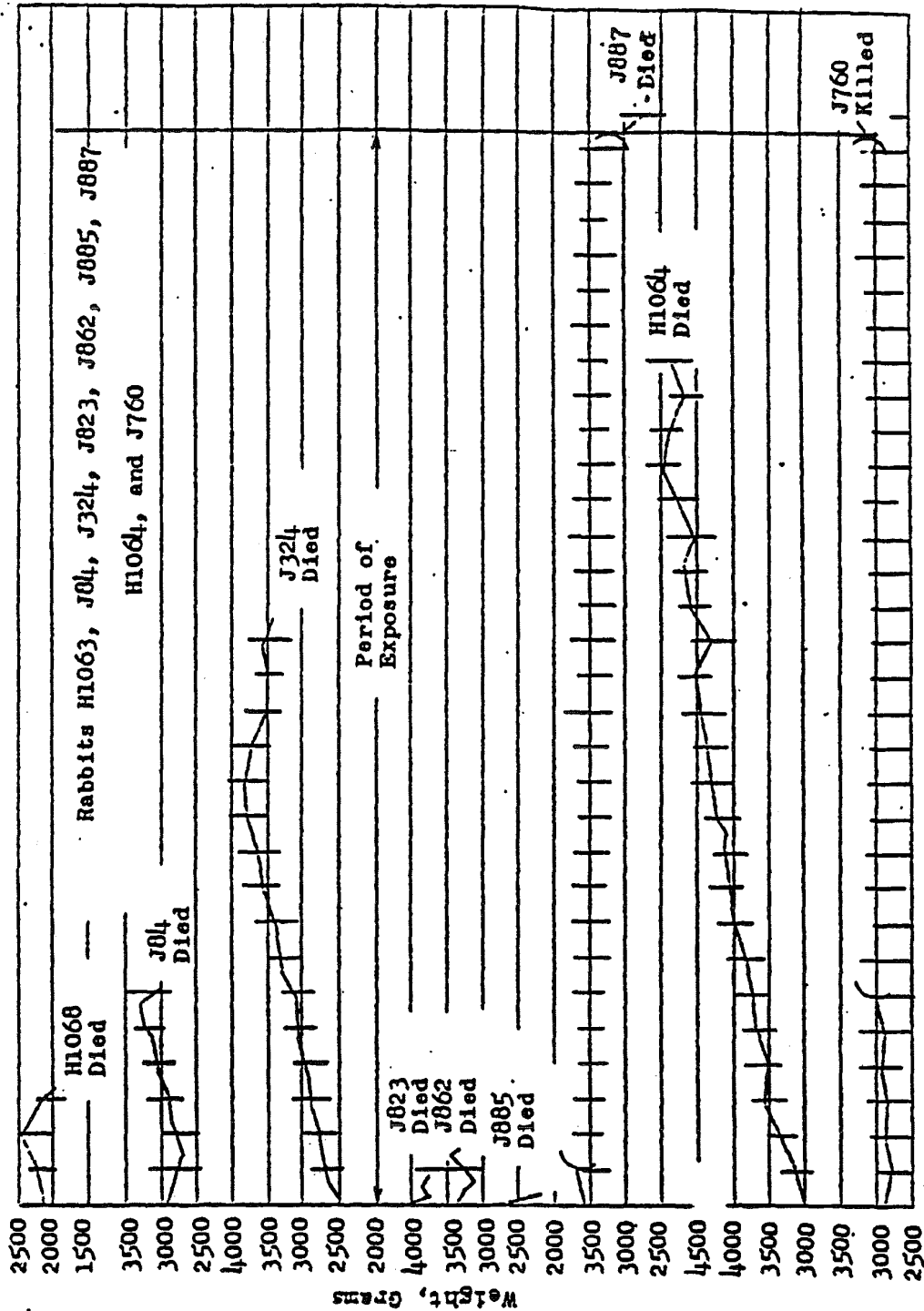
NEV 016690



Experiment No. 3
1.9 Micrograms of Aroclor 1242/liter

Figure 9

NEV 016691

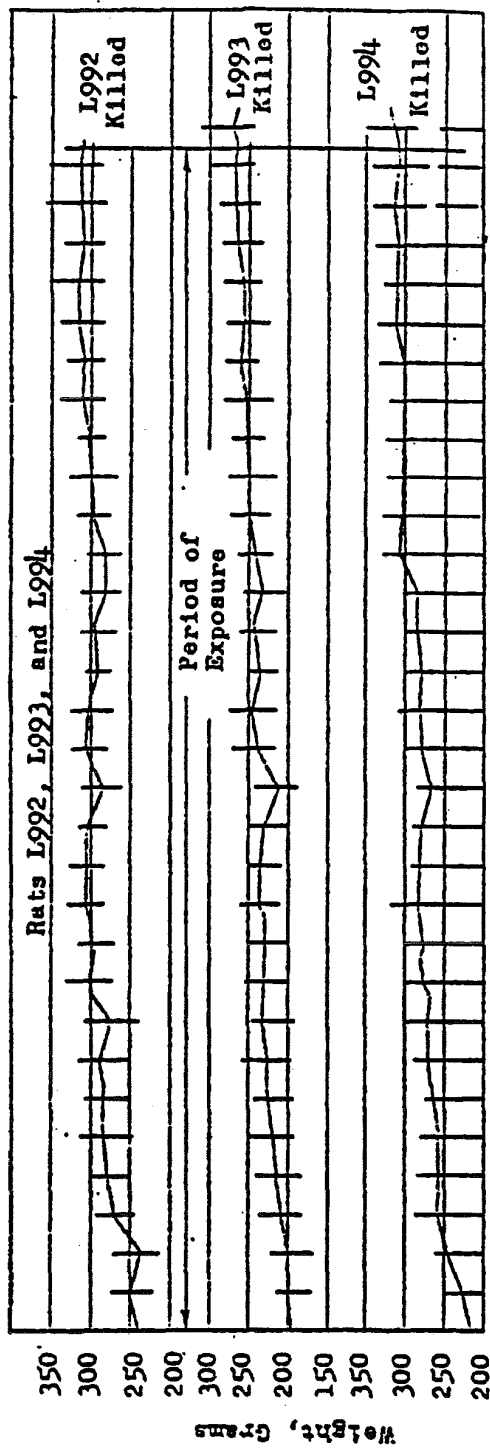


Experiment No. 3
1.9 Micrograms of Aroclor 1242/liter

Time, Weeks

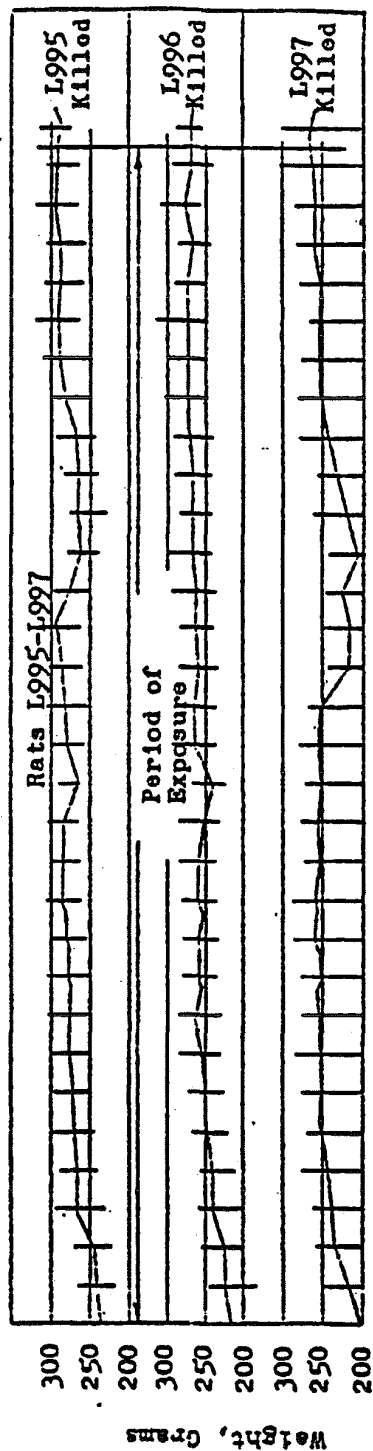
Figure 10

NEV 016692



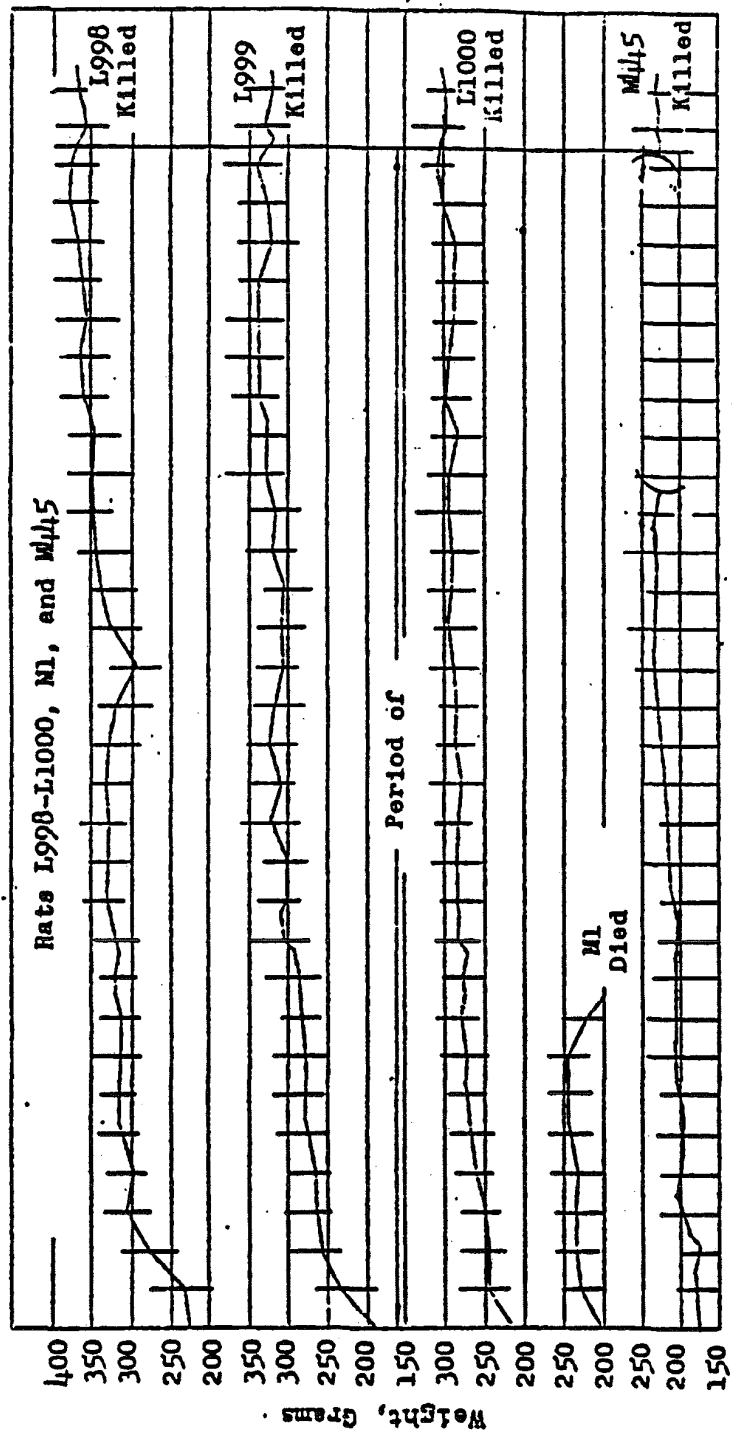
Experiment No. 3
1.9 Micrograms of Aroclor 1242/liter
Figure 11

NEV 016693



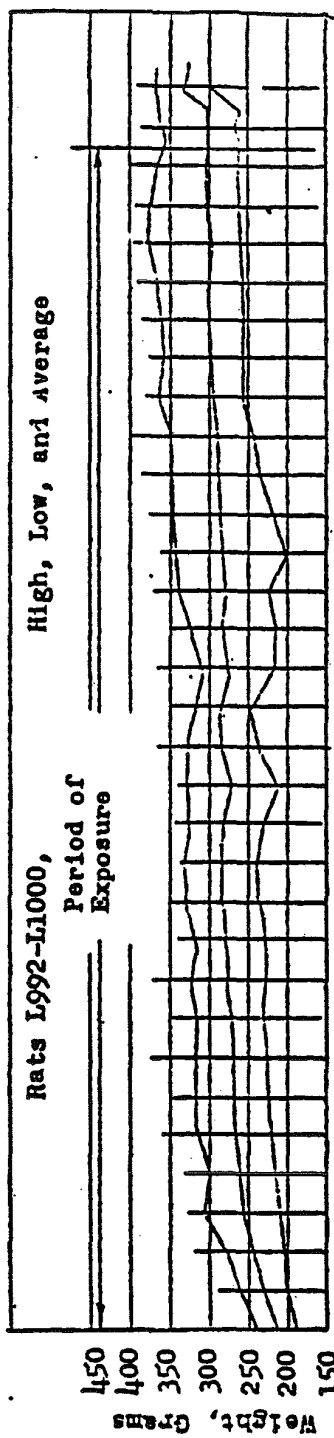
Experiment No. 3
1.9 Micrograms of Aroclor 1242/liter
Figure 12

NEV 016694



Experiment No. 3
1.9 Micrograms of Aroclor 1242/liter
Figure 13

NEV 016695

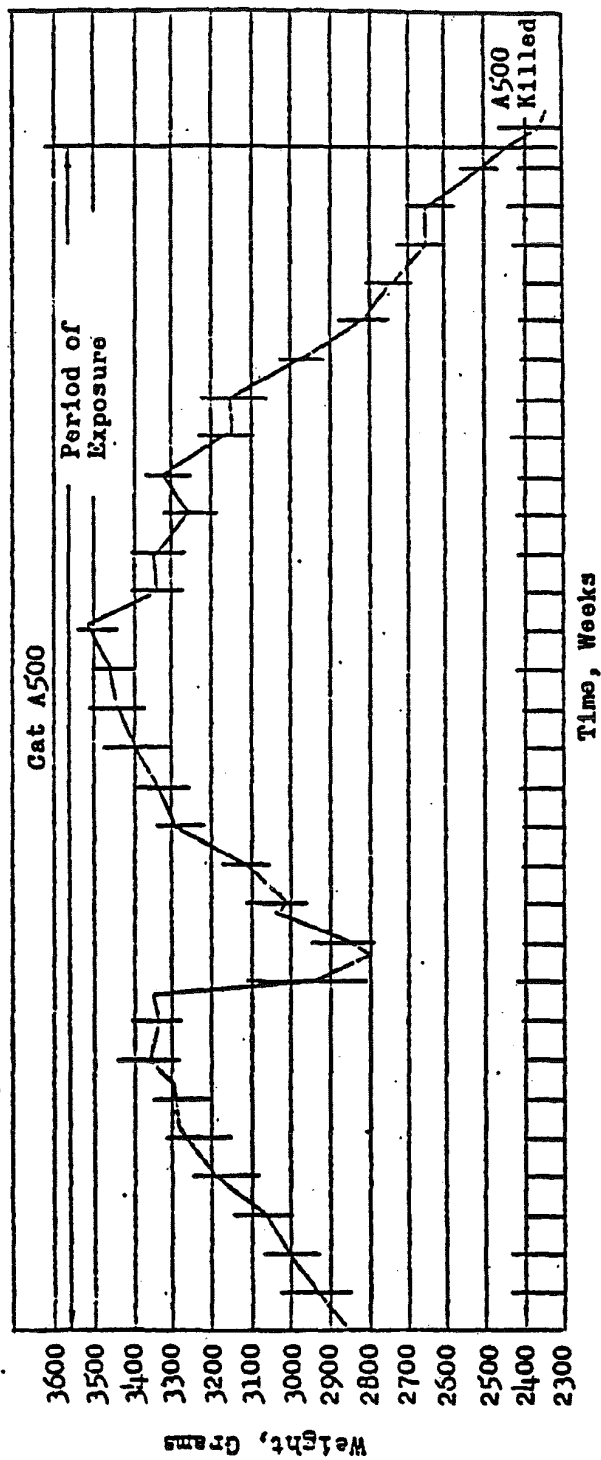


Time, Weeks

Experiment No. 3
1.9 Micrograms of Aroclor 1242/liter

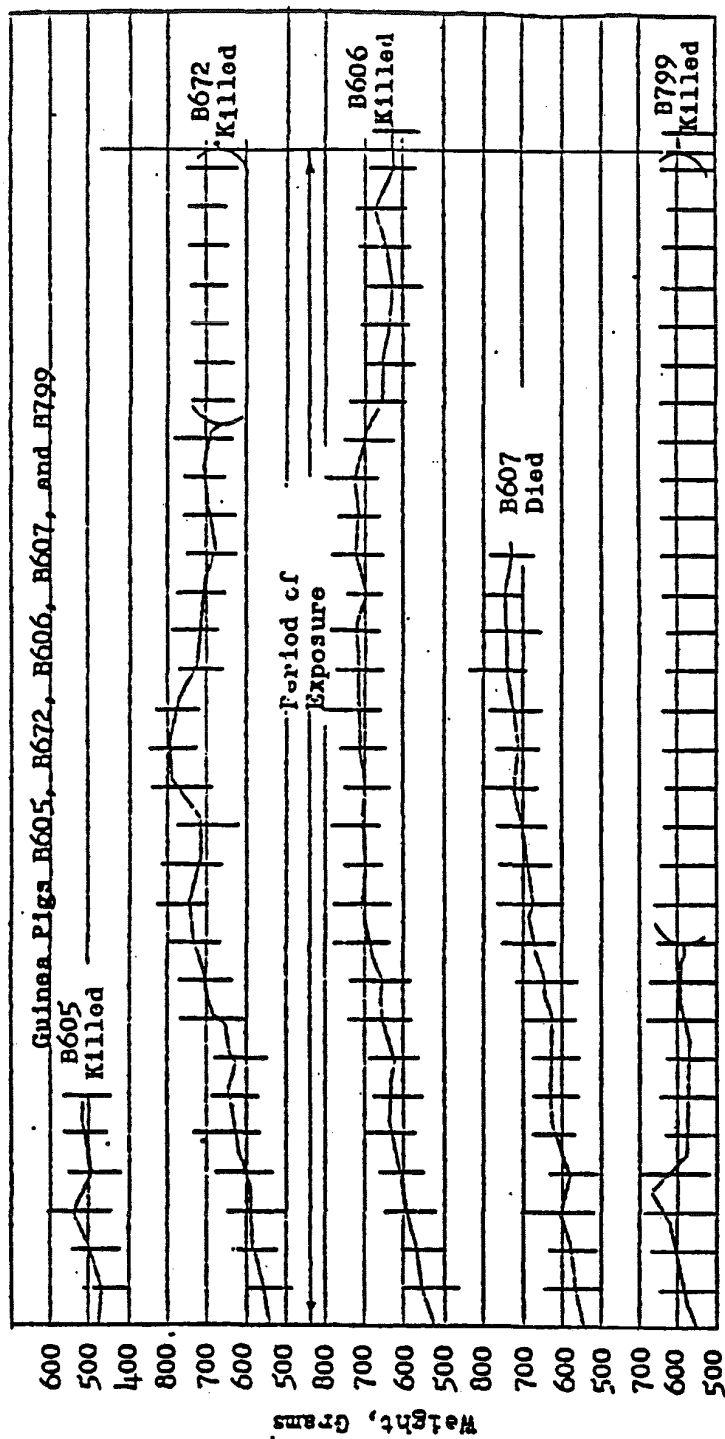
Figure 14

NEV 016696



Experiment No. 2
1.5 Micrograms of Aroclor 1254/litor Figure 15

NEV 016697

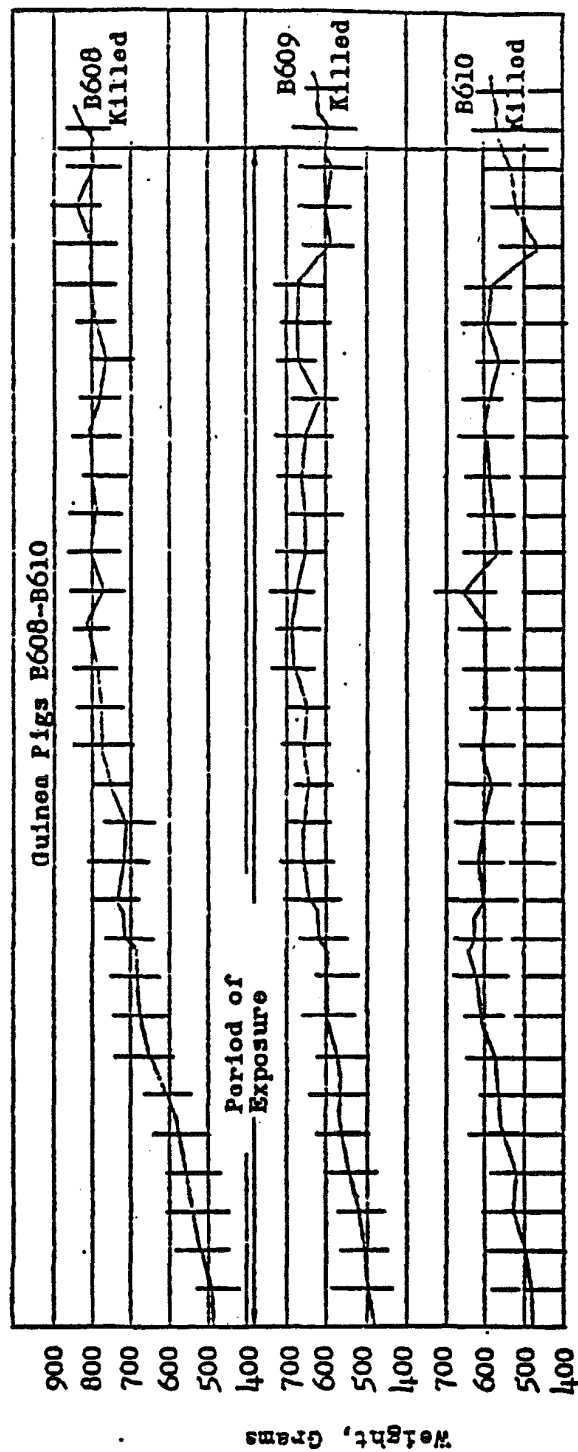


Time, Weeks

Experiment No. 2
1.5 Micrograms of Aroclor 1254/liter

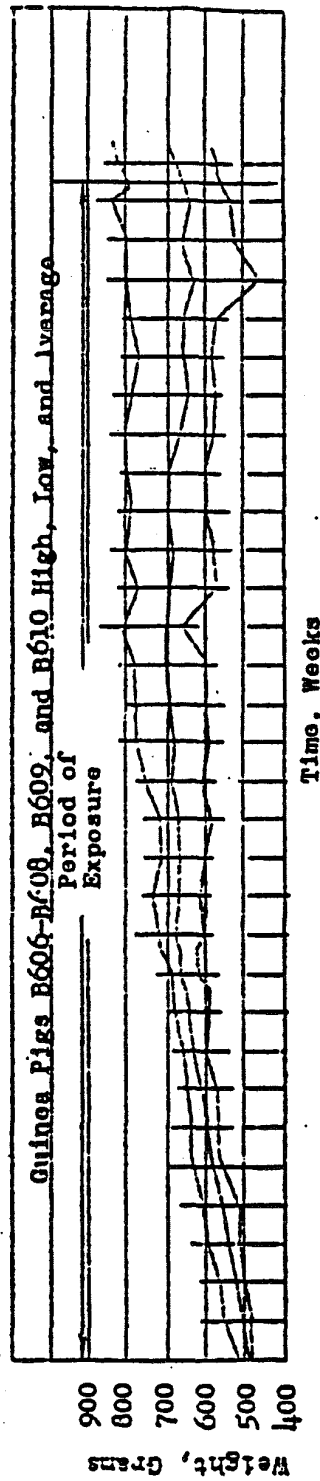
Figure 16

NEV 016698



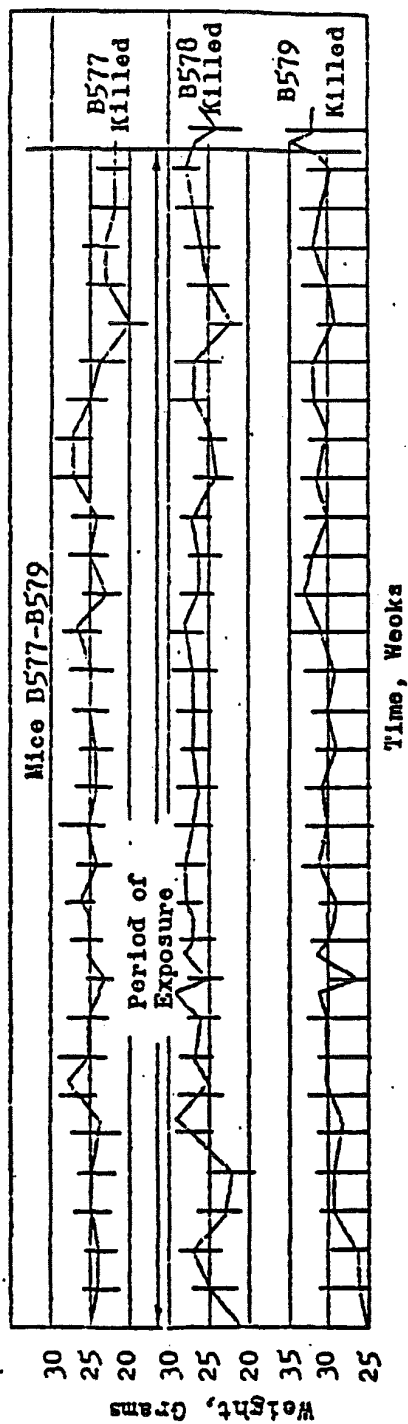
Experiment No. 2
 1.5 Micrograms of Aroclor 1254/liter
 Figure 17

NEV 016699



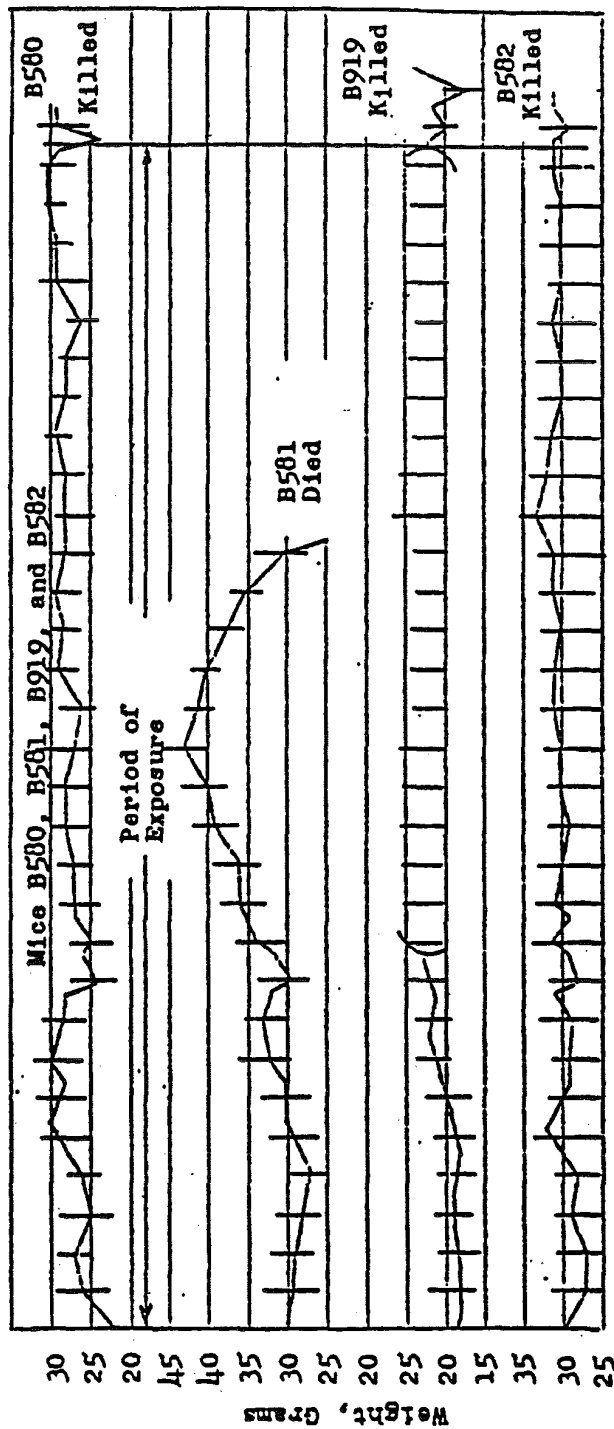
Experiment No. 2
1.5 Micrograms of Aroclor 1254/liter
Figure 18

NEV 016700



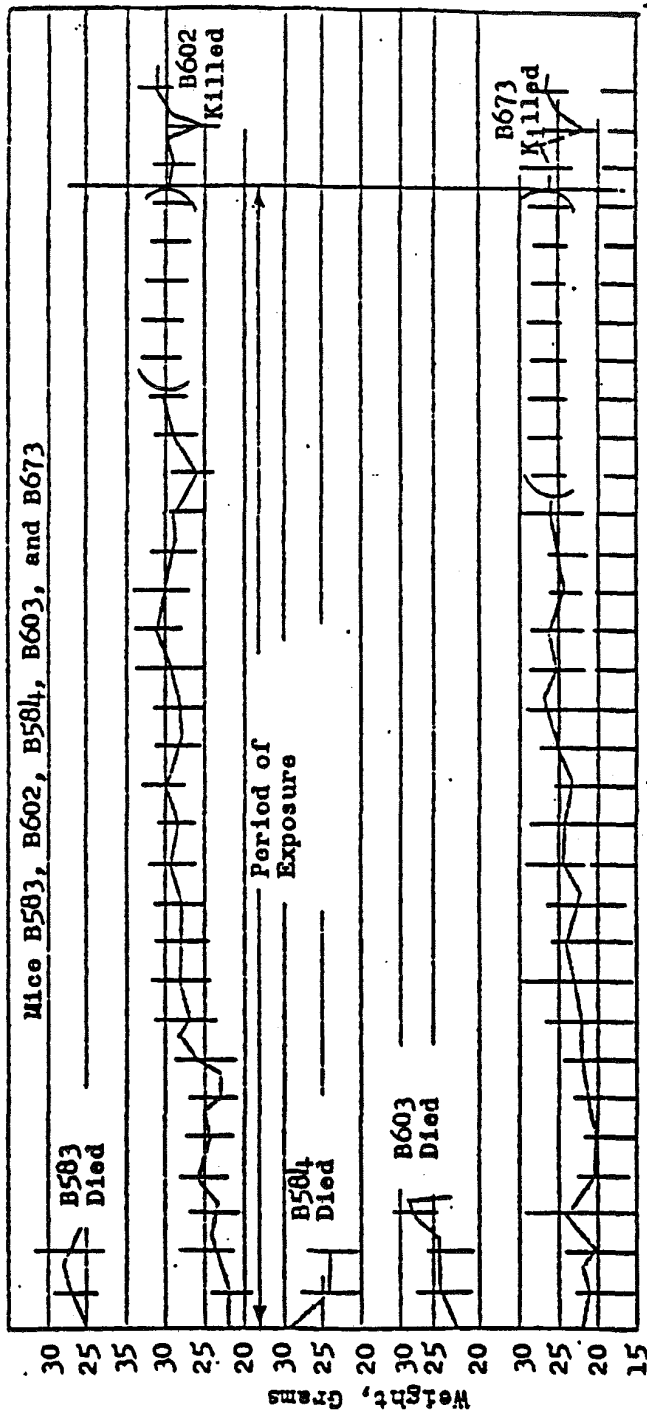
Experiment No. 2
1.5 Micrograms of Aroclor 1254/liter
Figure 19.

NEV 016701



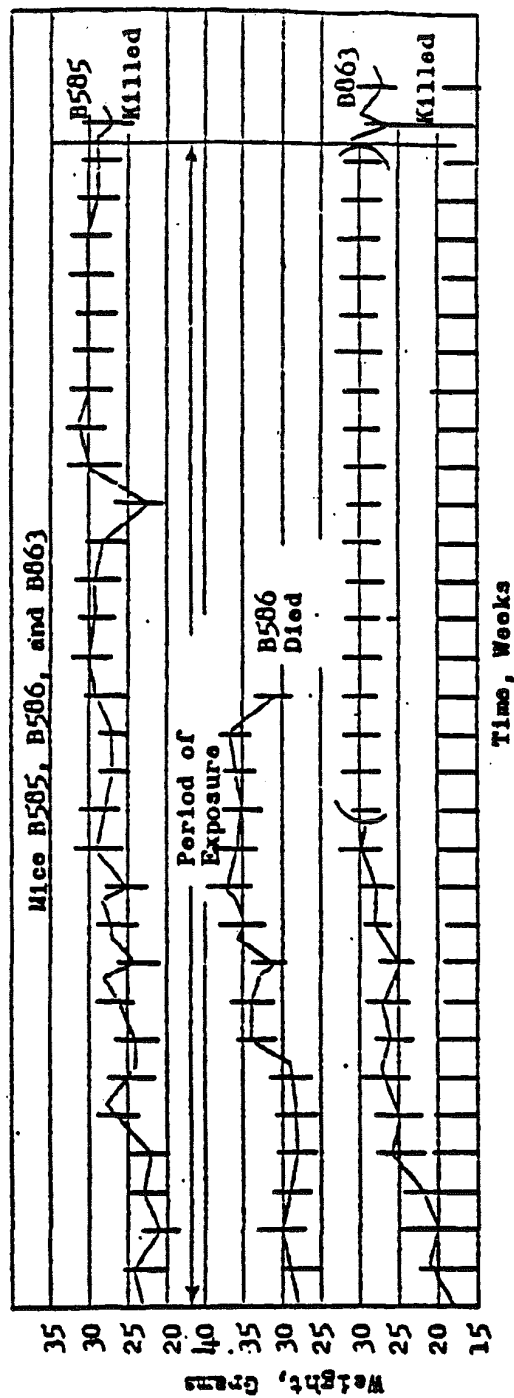
Experiment No. 2
1.5 Micrograms of Aroclor 1254/liter
Figure 20

NEV 016702



Experiment No. 2
1.5 Micrograms of Aroclor 1254/liter
Time, Weeks
Figure 21-a

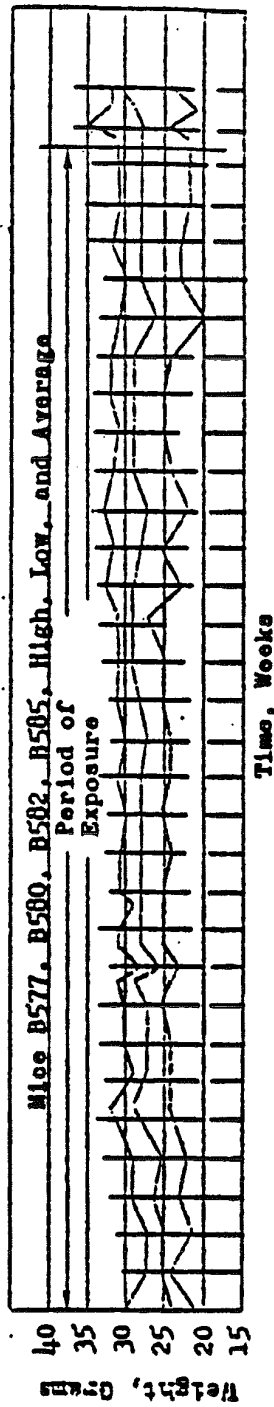
NEV 016703



Experiment No. 2
1.5 Micrograms of Aroclor 1254/liter

Figure 21-b

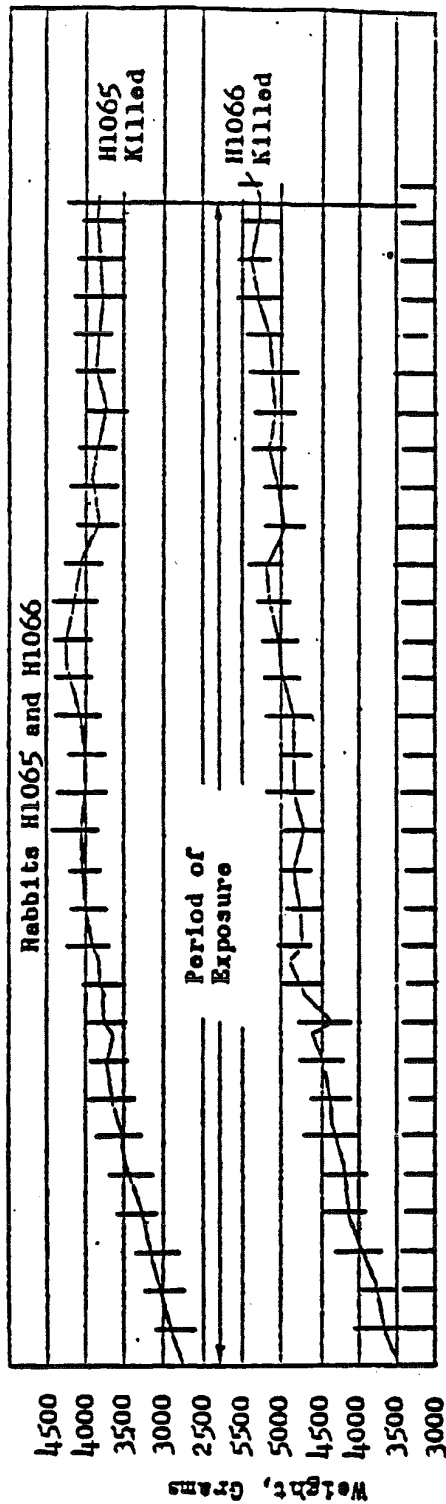
NEV 016704



Time, Weeks

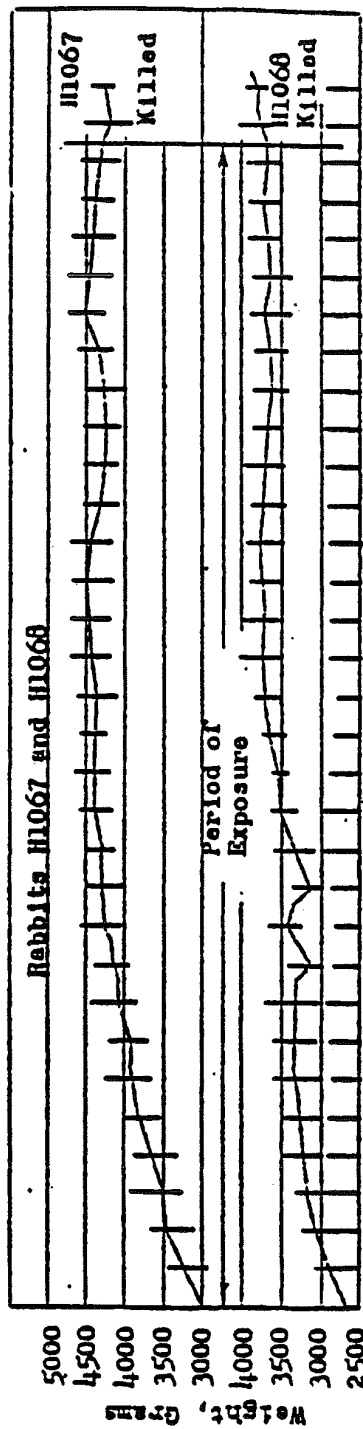
Experiment No. 2
1.5 Micrograms of Aroclor 1254/liter
Figure 22

NEV 016705



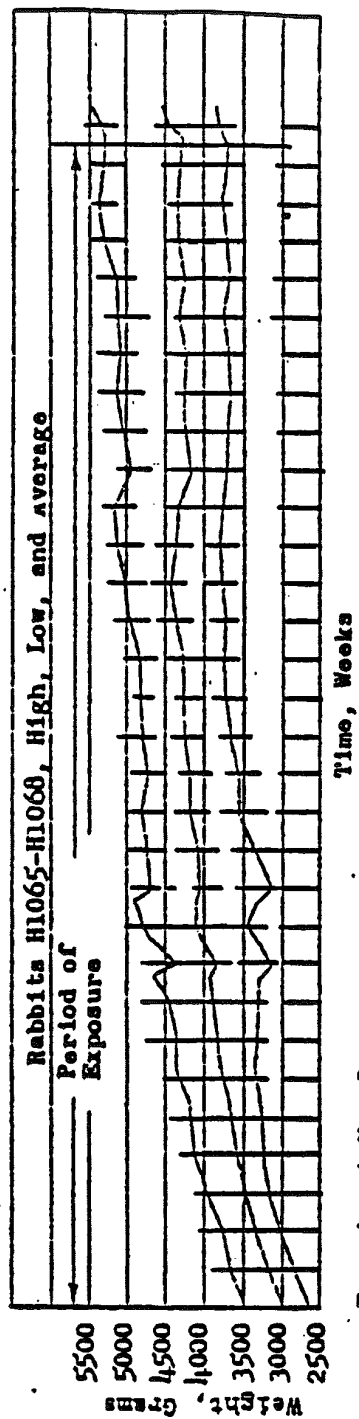
Experiment No. 2
1.5 Micrograms of Aroclor 1254/liter **Figure 23**

NEV 016706



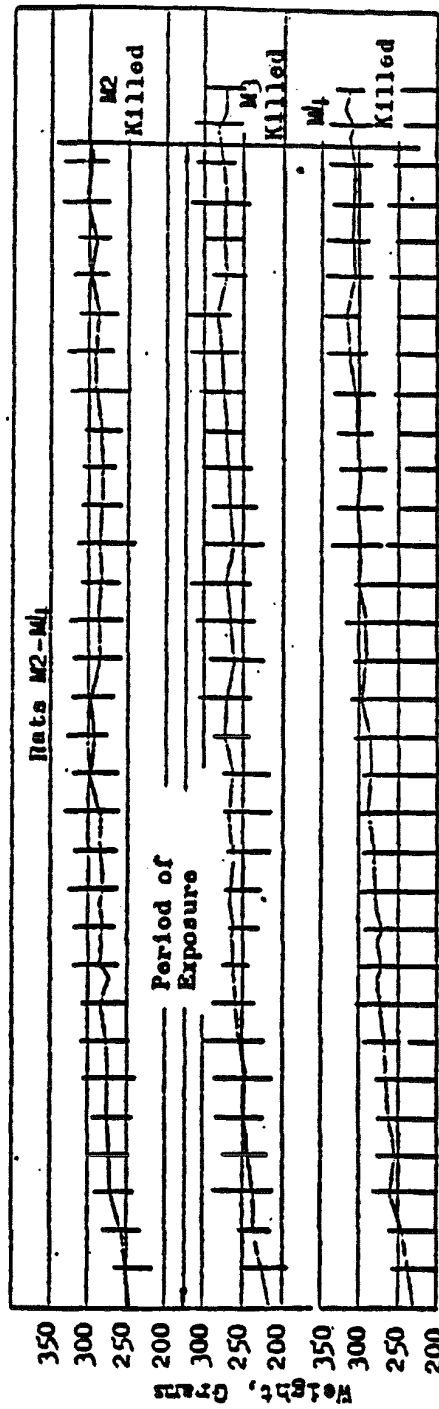
Experiment No. 2
 1.5 Micrograms of Aroclor 1254/liter
 Figure 24

NEV 016707



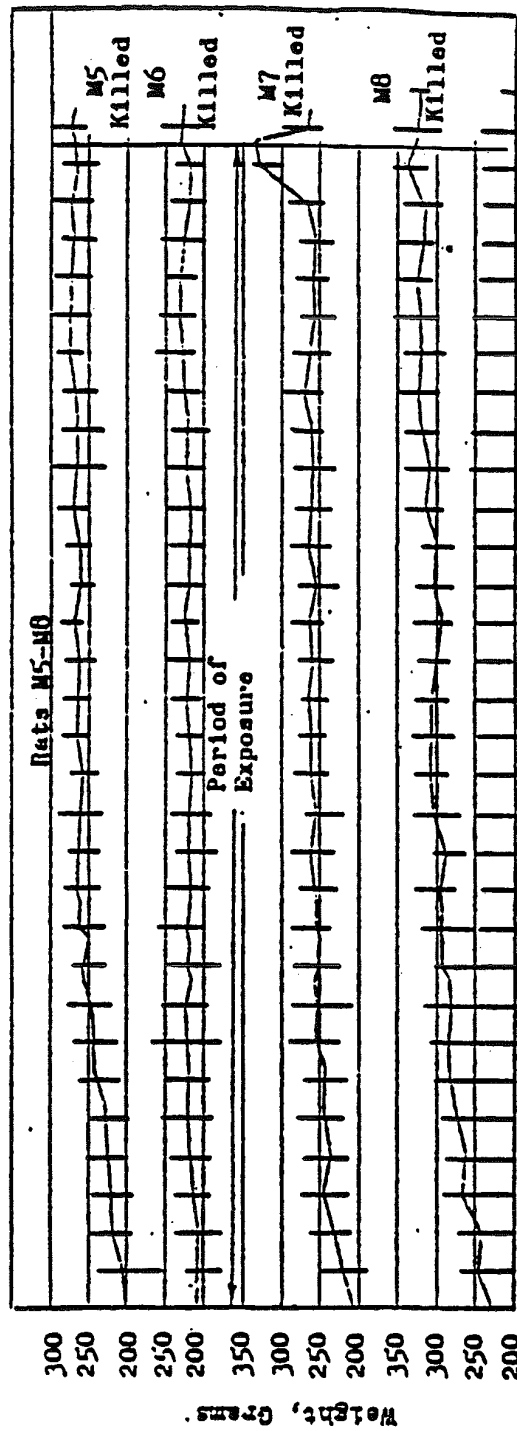
Experiment No. 2
1.5 Micrograms of Aroclor 1254/liter
Figure 25

NEV 016708



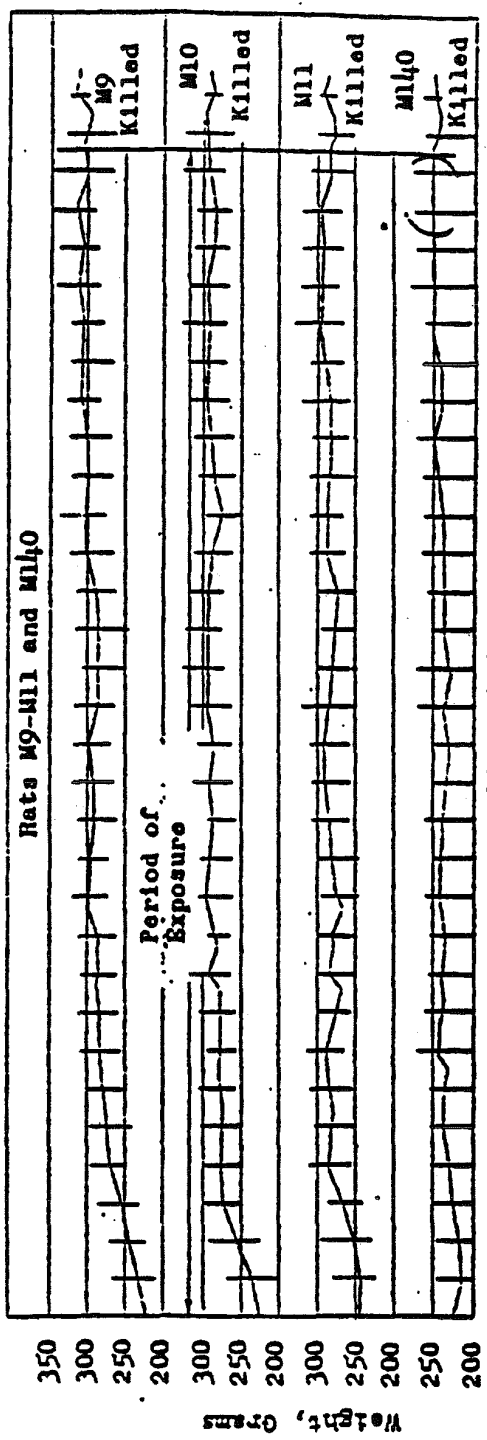
Experiment No. 2
1.5 Micrograms of aerosol 1254/liter
Figure 26

NEV 016709



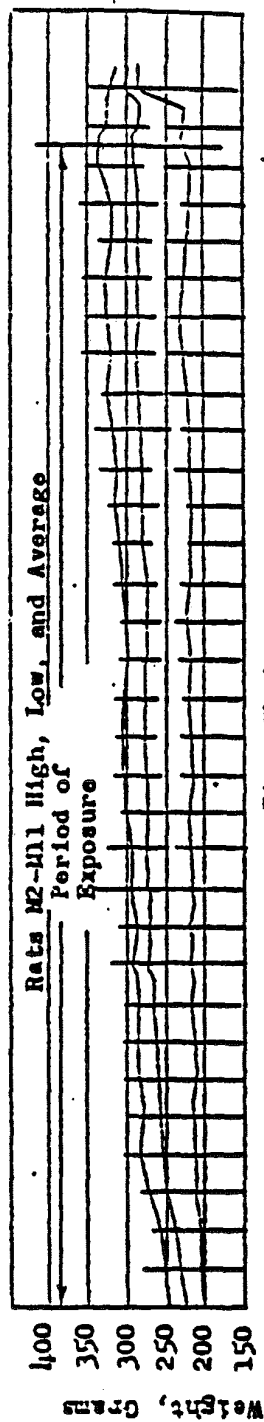
Experiment No. 2
 1.5 Micrograms of Aroclor 1254/liter
 Figure 27

NEV 016710



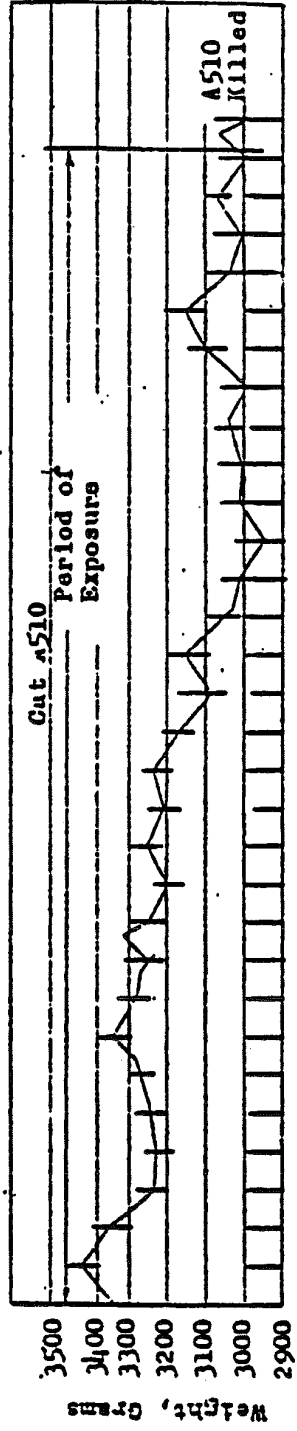
Experiment No. 2
1.5 Micrograms of aroclor 1254/liter
Figure 20

NEV 016711



Experiment No. 2
1.5 Micrograms of Aroclor 1254/liter
Figure 29

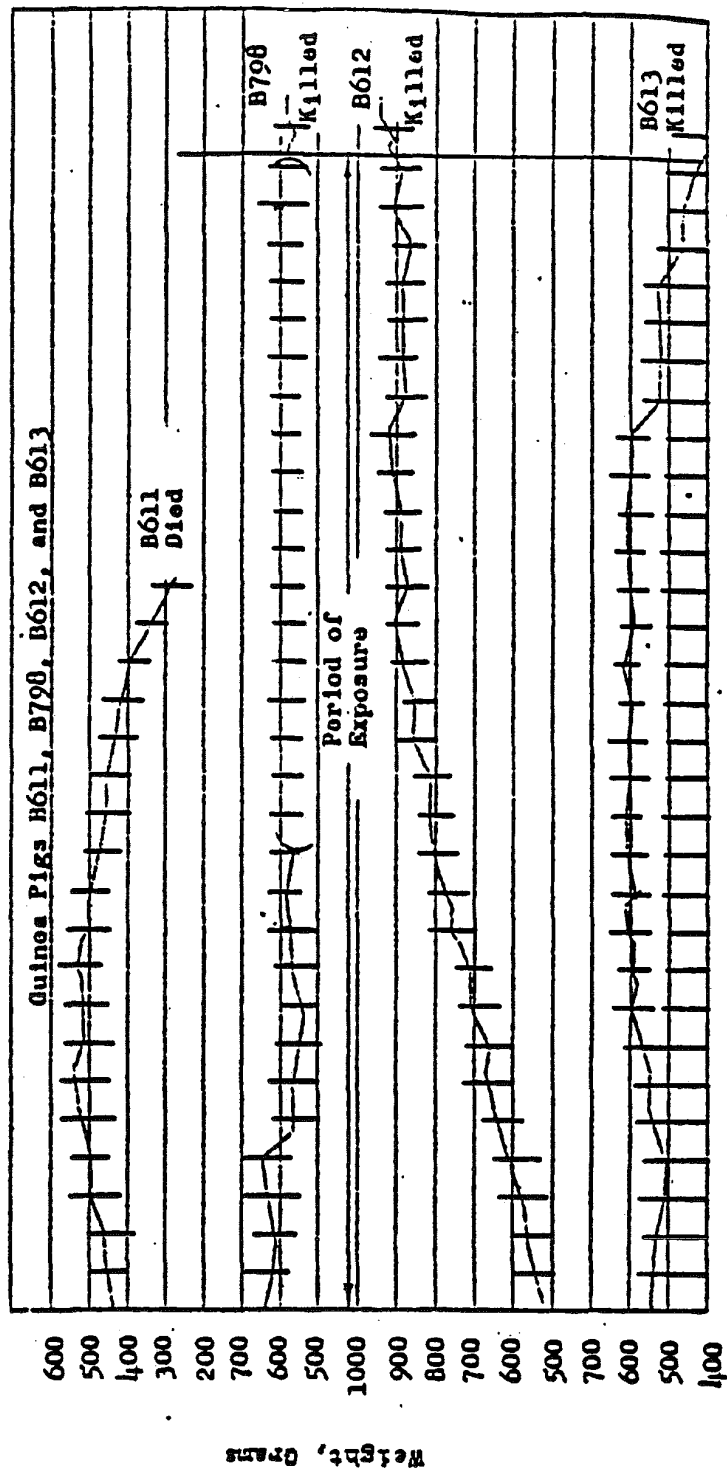
NEV 016712



Time, Weeks

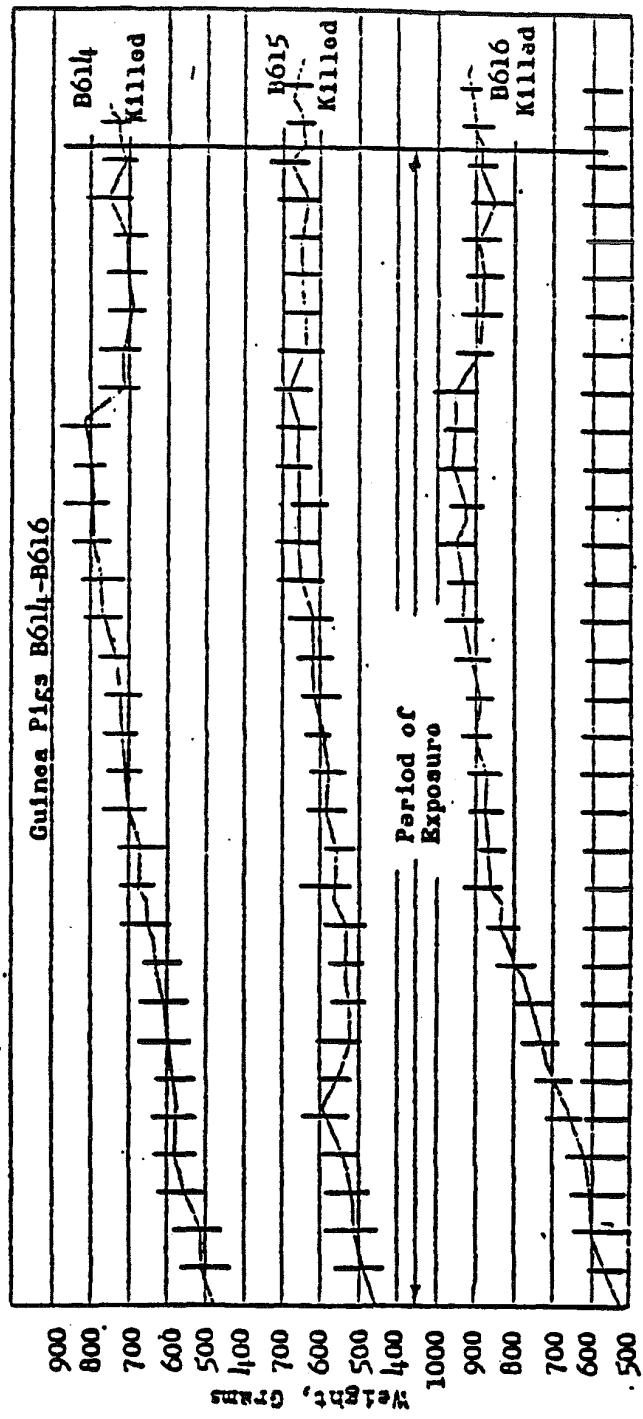
Experiment No. 2
Controls Subjected to Conditioned Air
Figure 30

NEV 016713



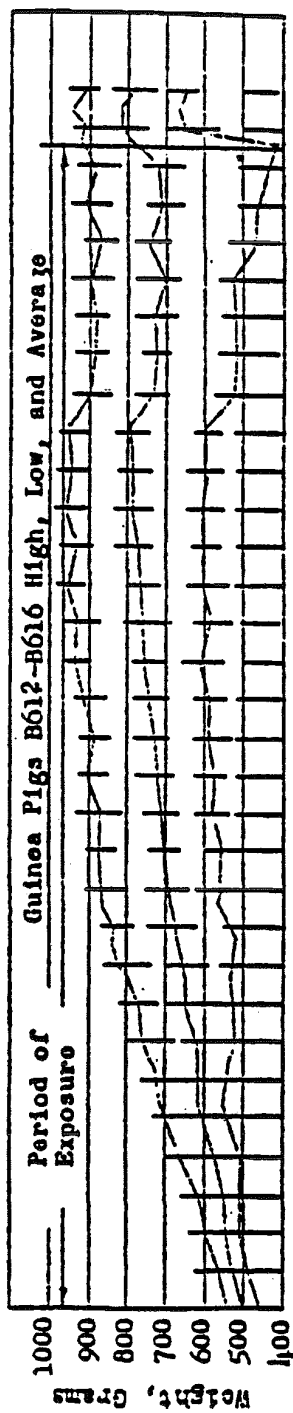
Experiment No. 2
Controls Subjected to Conditioned Air
Figure 31

NEV 016714



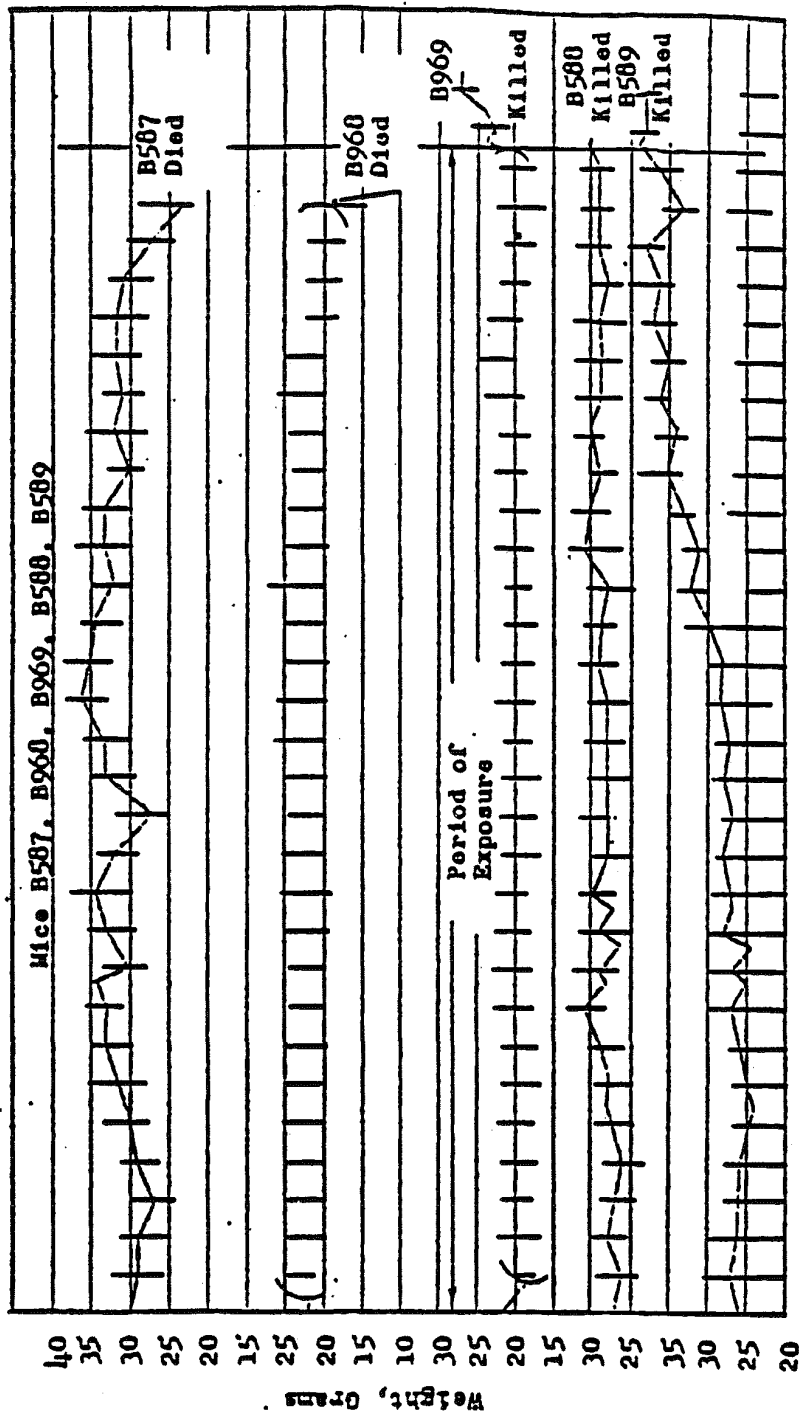
Experiment No. 2
Controls Subjected to Conditioned Air Figure 32

NEV 016715



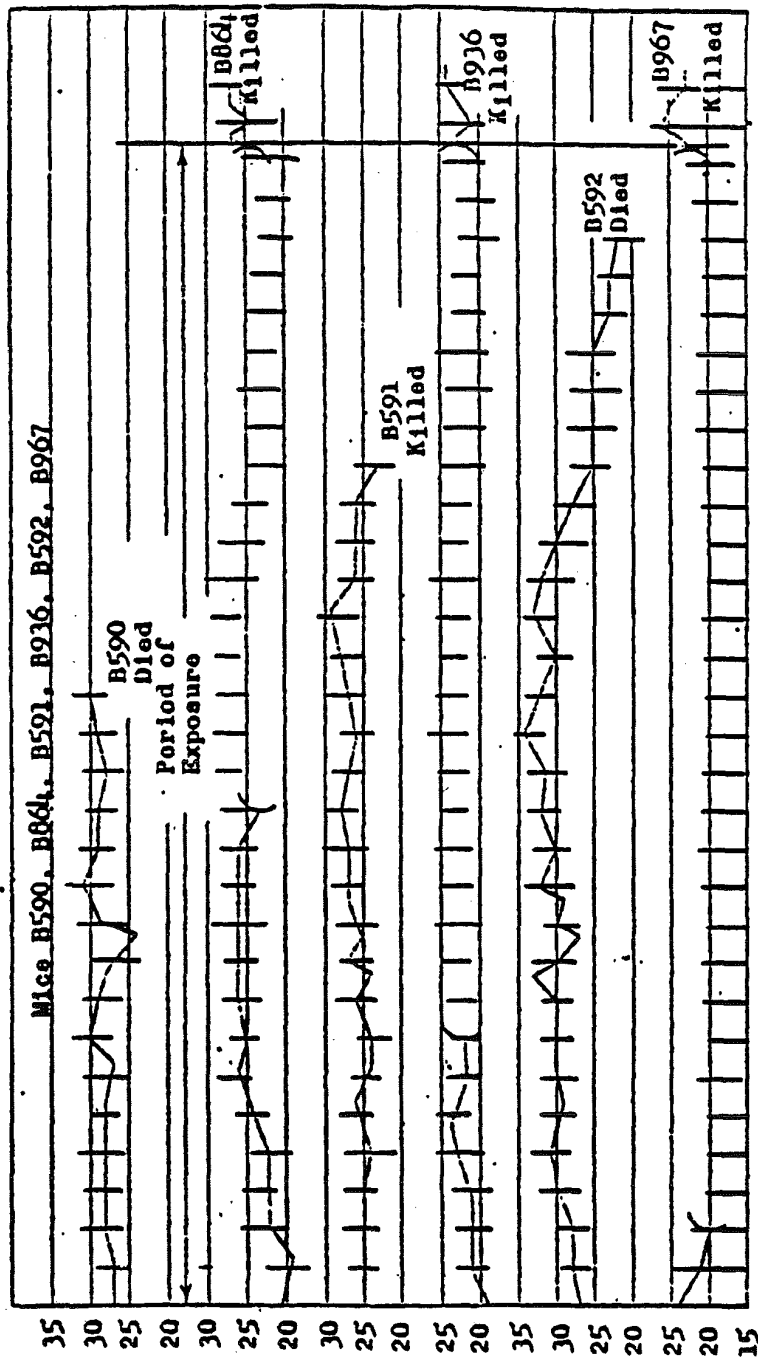
Experiment No. 2
 Controls Subjected to Conditioned Air
 Figure 33

NEV 016716



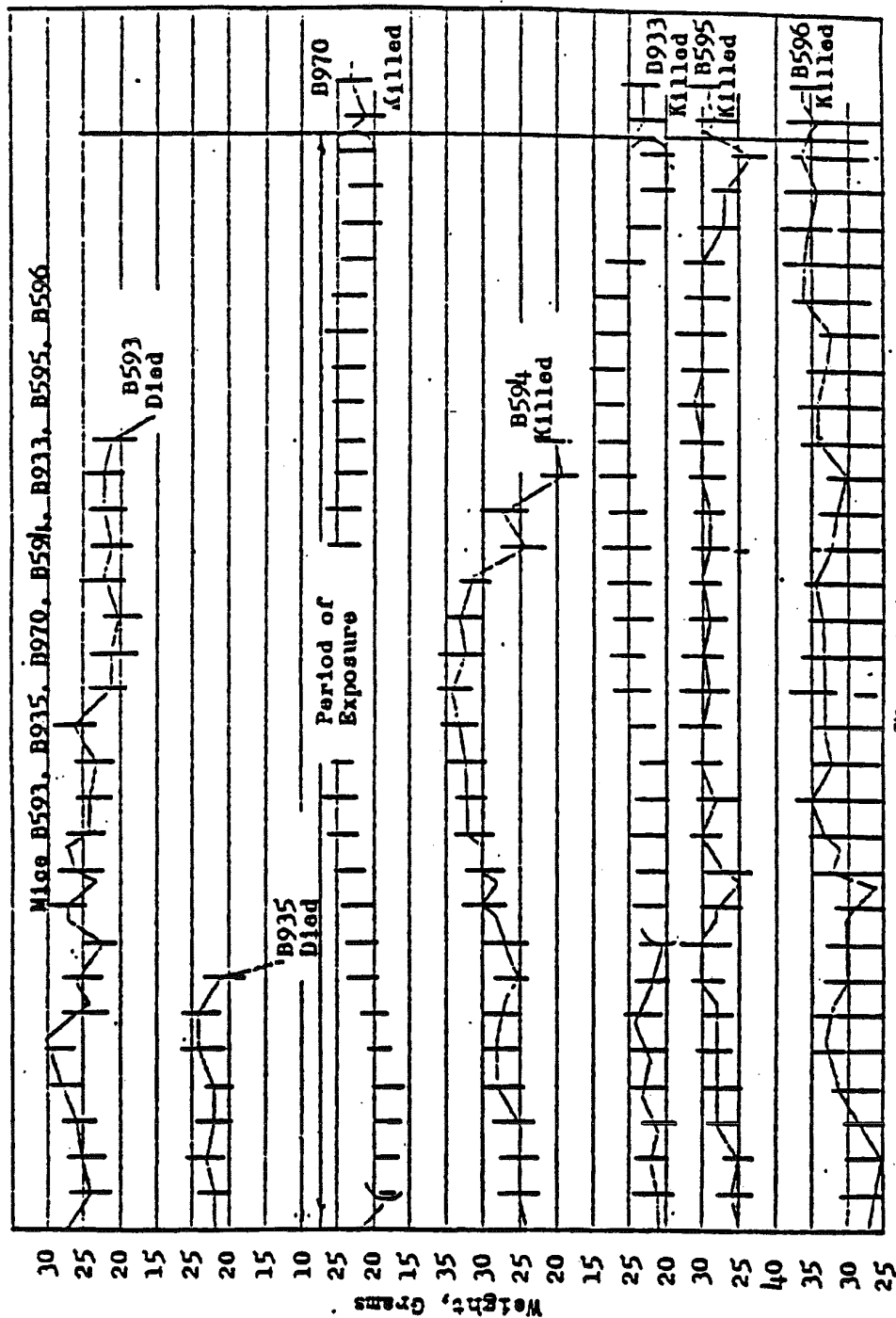
Experiment No. 2
Controls Subjected to Conditioned Air
Figure 3/4

NEV 016717



Experiment No. 2
 Controls Subjected to Conditioned Air
 Figure 35

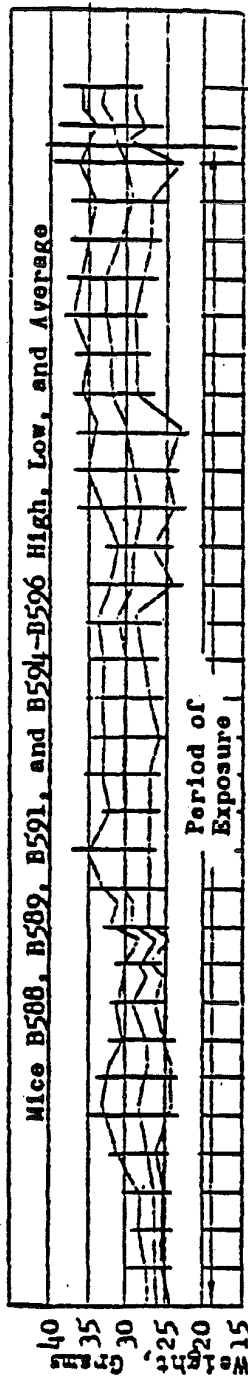
NEV 016718



Experiment No. 2
Controls Subjected to Conditioned Air

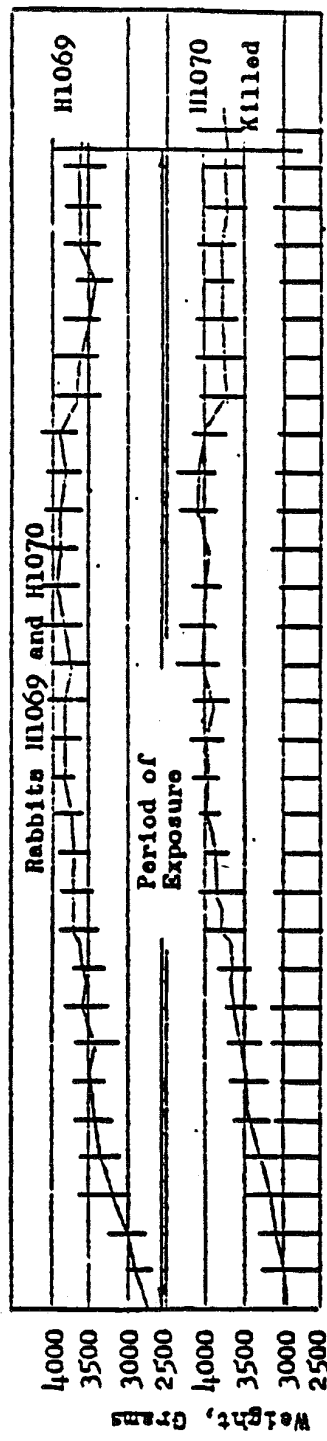
Figure 36

NEV 016719



Experiment No. 2
Controls Subjected to Conditioned Air
Figure 37

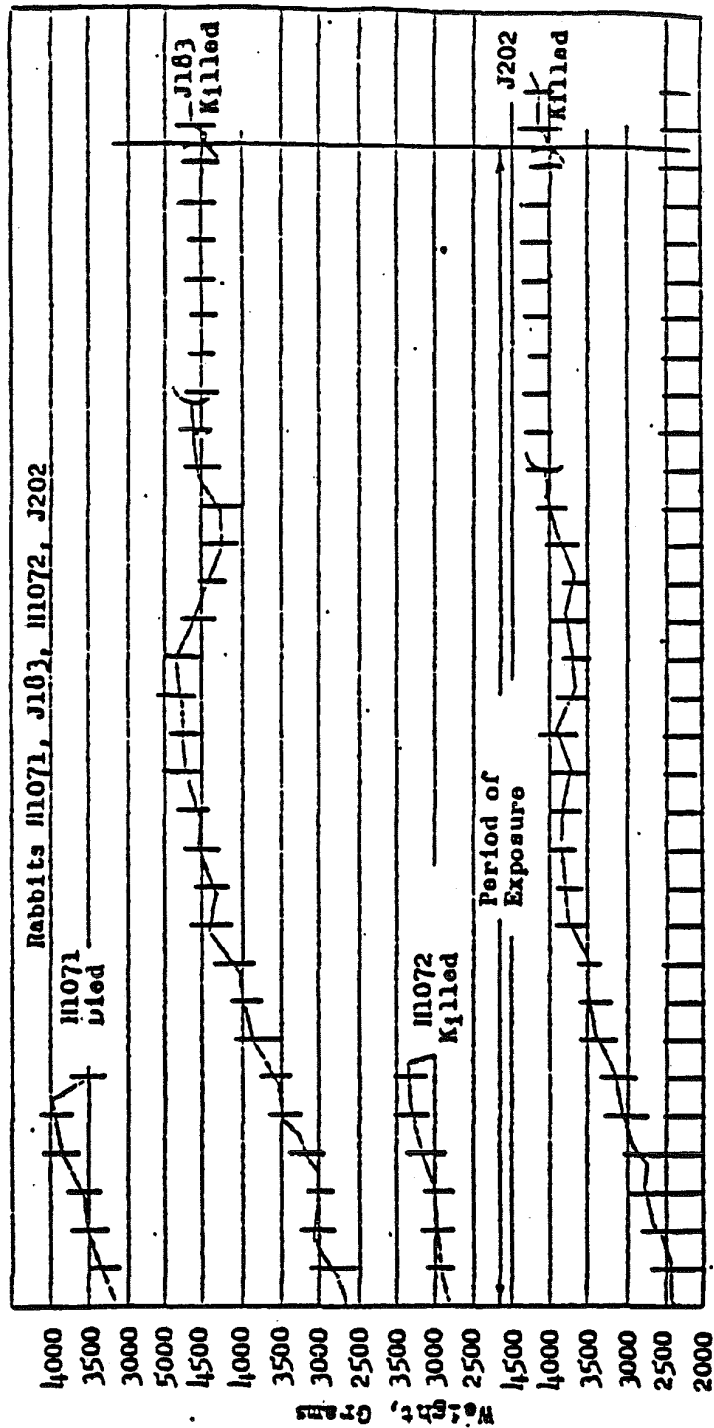
NEV 016720



Time, Weeks

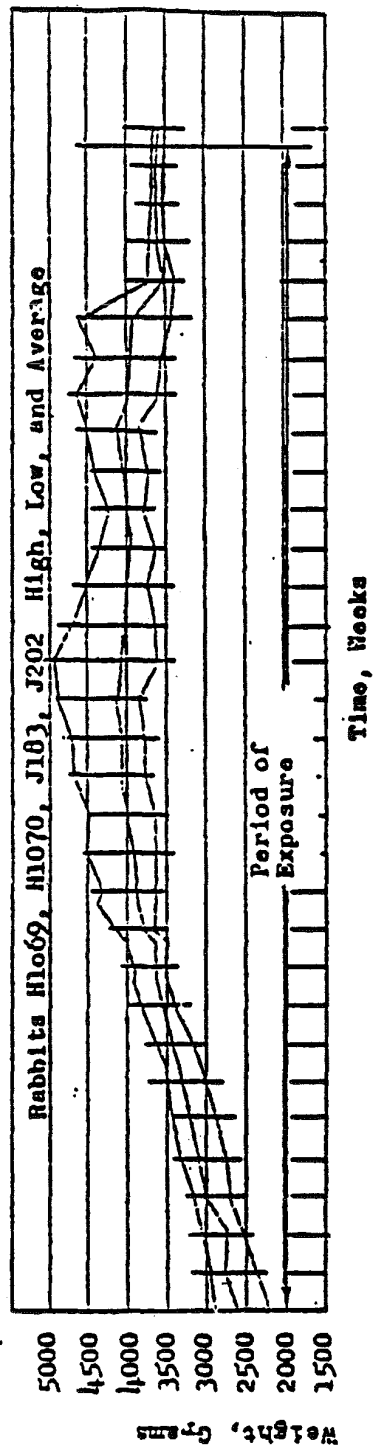
Experiment No. 2
Controls Subjected to Conditioned Air
Figure 30

NEV 016721



Experiment No. 2
Controls Subjected to Conditioned Air
Figure 39

NEV 016722

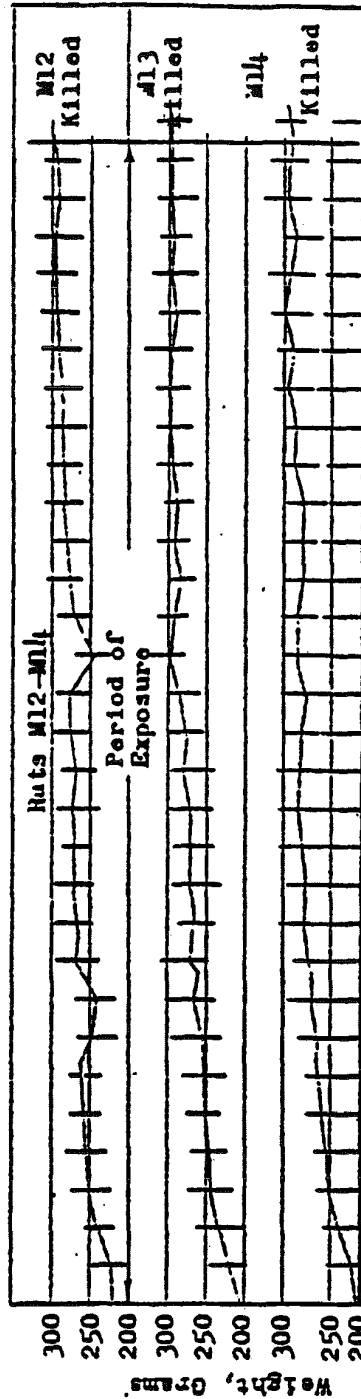


Time, Weeks

Experiment No. 2
Controls Subjected to Conditioned Air

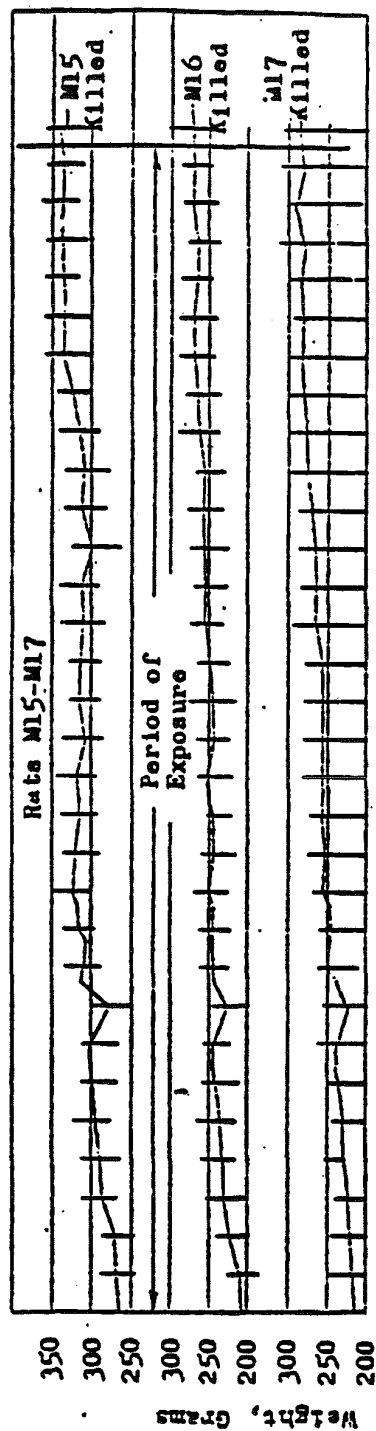
Figure 40

NEV 016723



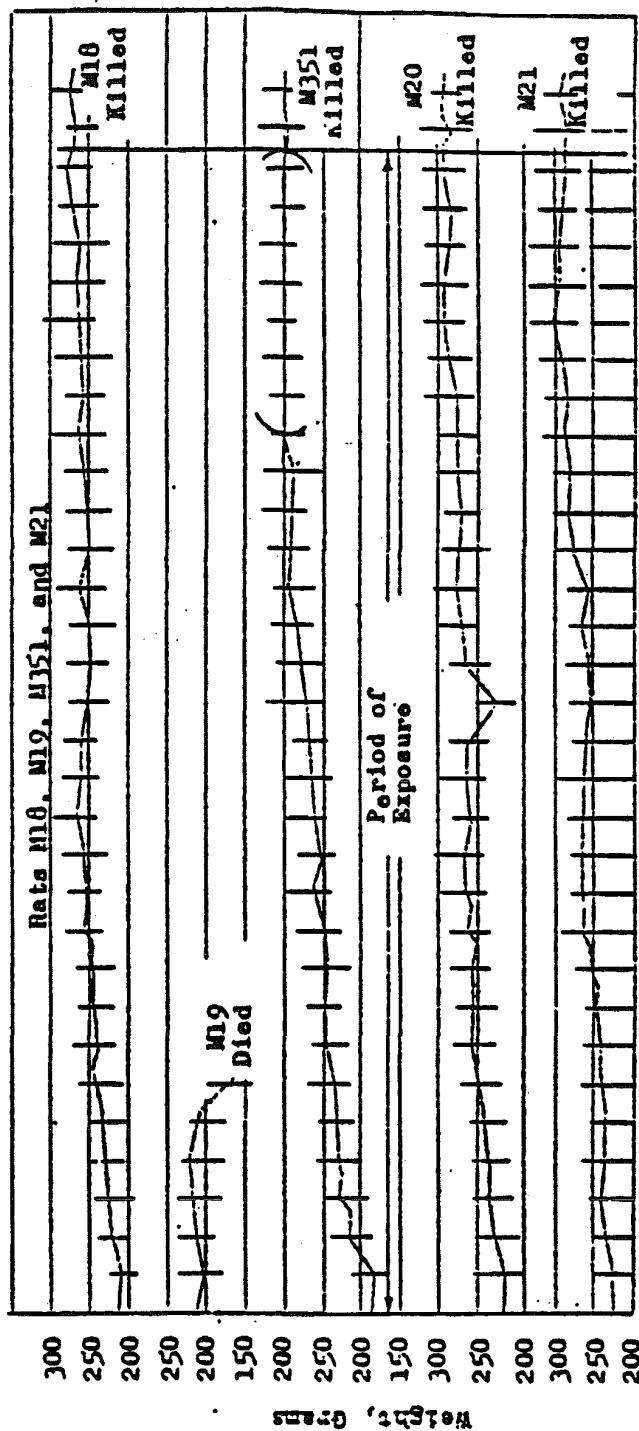
Experiment No. 2
Controls Subjected to Conditioned Air
Figure J41

NEV 016724



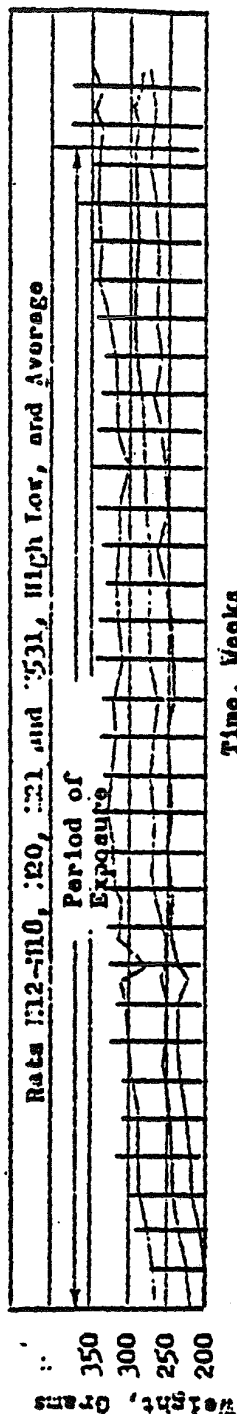
Experiment No. 2
Controls Subjected to Conditioned Air
Figure 4a

NEV 016725



Experiment No. 2
Controls Subjected to Conditioned Air
Figure 43

NEV 016726



Experiment No. 2
Controls Subjected to Conditioned air
Figure 44

NEV 016727

The Toxicity of the Vapors of Aroclor 1242[®] and Aroclor 1254[®]

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The Kettering Laboratory, Department of Preventive Medicine and Industrial Health
College of Medicine, University of Cincinnati

INVESTIGATION of the physiological response of animals to the inhalation of two Aroclors, namely 1242 and 1254,[®] was undertaken because in the earlier literature¹ dealing with the toxic effects of Aroclors, no distinction has been drawn between the toxic effects of chlorinated biphenyls and those of chlorinated naphthalenes, despite the differences in the chemical composition, physical characteristics, and industrial applications of these classes of compounds. In the case of the chlorinated biphenyls, at least, further toxicological investigation and consideration were required to demonstrate the relationship between the extent of their chlorination and their toxicity.

Properties

AROCLOR 1242 is a light, straw-colored, mobile liquid. According to Benignus, of Monsanto Chemical Company, it contains $42.0 \pm 0.5\%$ of chlorine, an amount which corresponds to a chlorinated biphenyl with three chlorine atoms in unassigned positions. The physical and chemical properties are given in Monsanto Application Bulletin No. O-P-115. Its specific gravity at 25°/25°C is 1.378 to 1.388; it has a distillation range of 325° to 360°C; a refractive index (D-line at 20°C) of 1.627 to 1.629; a Saybolt Universal viscosity at 100°F of 80 to 93 seconds, and a flash point (Cleveland Open Cup) of 176°-180°C. Its vapor pressure is about 4 mm at 150°C and about 30 mm at 200°C. Although insoluble in water and glycerine, it is soluble in most organic substances. At 745 mm of mercury and 25°C, the concentration of 1 mg of the vapor of

Aroclor 1242 per liter of air is equivalent to 96.9 ppm by volume.

Aroclor 1254, which corresponds to pentachlorobiphenyl, is a light, straw-colored, viscous liquid. The positions of the chlorine atoms have not been established. The molecular weight of pentachlorobiphenyl is 326.445, of which 84.3% is chlorine. Benignus has reported that Aroclor 1254 contains $55.0 \pm 0.5\%$ of chlorine. Bulletin No. O-P-115 describes Aroclor 1254 as having a specific gravity at 25°/25°C of 1.538 to 1.548; a distillation range of 365° to 390°C; a refractive index (D-line at 20°C) of 1.639 to 1.641, and a Saybolt Universal viscosity at 100° F of 1,800 to 2,500 seconds. The vapor pressure at 150°C is about 1.3 mm, and at 200°C is about 9 mm. The material is soluble in most organic substances, but it is insoluble in water and glycerine. At 745 mm of mercury and 25°C the concentration of 1 mg of Aroclor 1254 per liter is equivalent to 76.5 ppm by volume.

Experimental Method

EXPOSURE TO THE VAPOR: In a preliminary experiment (No. 1) with the vapor of Aroclor 1242, a group of animals was confined for seven hours on each of five days per week in a rectangular plywood chamber (volume 600 liters), of which the inner metal lining was coated with a baked chemically resistant plastic, through which was passed a stream of air laden with Aroclor 1242 in a known concentration. The stream of air, conditioned with respect to temperature (75° = 3°F inside the chamber), dust and humidity, entered the chamber through an "Anemostat," located at the center of its top, at the rate of 500 liters per minute, as measured by an inclined manometer attached

Presented at the Seventeenth Annual Meeting of the
AMERICAN INDUSTRIAL HYGIENE ASSOCIATION, Philadelphia,
April 26, 1956.

DEPOSITION
EXHIBIT

14

2-15-94

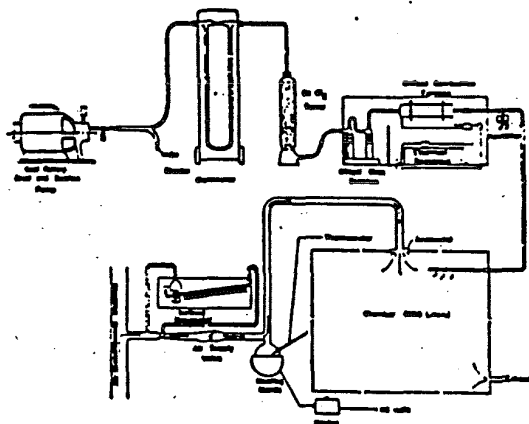


Fig. 1.

Schematic diagram of equipment for volatilizing Aroclor, chamber, and equipment for combustion and collection of samples.

to a venturimeter (Fig. 1). The air was withdrawn by suction from the chamber through an exit tube located on the rear wall near the floor, equidistant from the sides.

Aroclor 1242 was volatilized from a heated glass well maintained at 132° to 138°C. The air passed over the surface of the liquid before entering the chamber (Fig. 1).

In a second similar experiment performed on the same Aroclor (No. 2), the temperature of the liquid was kept at 100° to 105°C.

While the second experiment with Aroclor 1242 was in progress, an experiment involving the vapor of Aroclor 1254 (No. 1) was carried out in a second chamber similar to the first except for the absence of a plastic inner lining. The rate at which air flowed over liquid Aroclor 1254, which was maintained at 130° to 135°C, was 400 liters per minute.

In order to accustom the animals to the experimental procedure, they were kept in their respective chambers for seven hours on each of four consecutive days during the week prior to the introduction of the Aroclor vapor into the chamber. For purposes of control (No. 1), a third group of animals was confined, throughout a like period before and during the period of the exposure

to the Aroclors, in another chamber supplied only with conditioned air.

A second set of experiments with each of the Aroclors at lower concentrations was conducted in the same chambers. In the third experiment with Aroclor 1242, which was heated to 55° to 60°C, the air passed over the liquid at the rate of 800 liters per minute. In the second experiment with Aroclor 1254, the liquid was maintained between 115° and 125°C while 700 liters of air per minute passed over its surface before entering the chamber. The animals exposed in each of these experiments, as well as a second group of controls, were confined in separate 600 liter chambers during a seven hour period on each of five days per week during the week preceding the initial exposure of the test animals to the vapor of the respective Aroclors.

In all experiments, the period of exposure or confinement (controls) was seven hours per day on five days per week for several weeks. In all instances, the original group consisted of one cat, six guinea pigs, ten mice, four rabbits, and ten rats. Among both the experimental and control groups, several animals died from extraneous causes during the course of these experiments and were replaced very soon thereafter. In the second experiment with Aroclor 1242, in the first experiment with Aroclor 1254, and in the comparable group of controls, representative animals were killed throughout the experiment.

METHOD FOR THE DETERMINATION OF THE AROCLORS IN AIR: These materials were determined quantitatively by virtue of the fact that, on thermal decomposition, they yield hydrochloric acid which with silver nitrate, forms a suspension, the density of which could be measured by means of the Beckman spectrophotometer at 500 mμ.

On each day, two samples of air from each chamber were collected by passing air at the rate of one liter per minute (for 15 minutes in the case of the higher concentrations and for 45 minutes in case of the lower concentrations) through a fused silica combus-

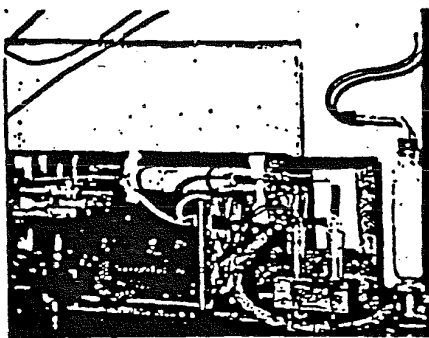


Fig. 2.
Humidifier, furnace, and sampling towers.

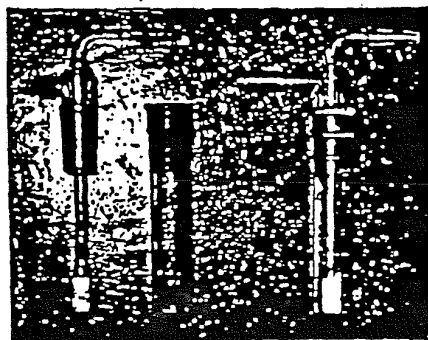


Fig. 3.
Sampling towers. (Midget Bubblers)

tion furnace, and then through two midget bubblers, in series, each containing 10 ml of 0.1 N sodium hydroxide. A quartz tube (13 mm outside diameter, 7 mm inside diameter and 16.25 inches in length) contained several strips of folded platinum foil. The middle nine inches of the tube were wrapped with a heating unit. The heating unit of the furnace (Fig. 2) consisted of 20 feet of B. and S., gauge 22, nichrome wire (1 ohm per foot), and was covered with asbestos cement. The sample of air was humidified by a cotton wick saturated with water placed 0.5 inch upstream from the quartz tube. With the furnace maintained at 850°C, the Aroclor was decomposed in the presence of H₂O to form HCl; this was absorbed in 0.1 N sodium hydroxide in midget bubblers (Fig. 3) equipped with

fritted-glass bubblers (Mine Safety Appliance Company No. 43867).

The 0.1 N sodium hydroxide was prepared by dissolving 4.0 g of pellets (ACS specifications 0.01% Cl) in about 50 ml of double-distilled water. To this were added 10 g of arsenic trioxide (chloride-free) dissolved in water and filtered through Whatman No. 42 filter paper. The combined solutions were diluted to 1,000 milliliters.

The sample was transferred to a 25 ml graduated cylinder to which one drop of a solution of phenolphthalein was added (1 g of phenolphthalein dissolved in 100 ml of CP methanol). The solution was neutralized with 3N nitric acid (190 ml CP concentrated nitric acid diluted to 1,000 ml with double-distilled water) and diluted to 23 ml with double-distilled water. One ml of 3N nitric acid was added to obtain a pH of 1. After adding 1 ml of a solution of silver nitrate (3 g of AgNO₃ diluted to 1,000 ml with double-distilled water), the suspension was mixed by inversion. After standing 30 minutes, the transmission of wave-length 500 mμ was measured in a 50 cm cell by means of a Beckman spectrophotometer which was set against a corresponding cell containing a reagent blank.

The amount of Aroclor was estimated by means of a standardized curve prepared from known quantities of sodium chloride. A curve presenting the transmission values at 500 mμ of suspensions prepared from sodium chloride ranging from 5.0 to 120 micrograms per 25 ml of final suspension is shown in Fig. 4. On the basis of 42.0% of chlorine in Aroclor 1242 and 55.0% in Aroclor 1254, one microgram of sodium chloride is equivalent to 1.442 micrograms of Aroclor 1242 or to 1.1028 micrograms of Aroclor 1254.

Experimental Results

MORTALITY—AROCLOR 1242: No signs of intoxication were observed in any of the members of a group of 31 animals (Experiment No. 1), all of which survived throughout a period of 24 days, on 17 of which they were subjected to the inhalation of air bearing 8.6 micrograms of Aroclor 1242 per liter (0.83 ppm) for seven hours (Table I).

One cat, four guinea pigs, six mice, two rabbits, and eight rats survived without

signs of intoxication for seven days over a air bearing 1242 in the micrograms (certain of guinea pigs bit, and two to fewer pe Table I). Transverse exposed animals those which corresponded to No. 1, Tamice, three rabbits, and following 1 hours on 1 of 122 da which the dust, humidity vapor o vora (three bit, and to periods of

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Aroclor 1254
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Time of the 2
Time of the 3

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Aroclor 125
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Time of the 2
Time of the 3

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signs of intoxication throughout their exposure (Experiment No. 2) for seven hours on each of 82 days over a period of 120 days to air bearing the vapor of Aroclor 1242 in the concentration of 6.83 micrograms per liter (0.66 ppm). Certain other survivors (two guinea pigs, three mice, one rabbit, and two rats) were subjected to fewer periods of exposure (cf. Table I). The deaths from extraneous causes among the exposed animals were fewer than those which occurred among the corresponding group of controls (No. 1, Table I). One cat, three mice, three guinea pigs, three rabbits, and eight rats survived following confinement for seven hours on 84 days over a period of 122 days, in a chamber in which the air, conditioned with respect to dust, humidity and temperature, contained no vapor of either Aroclor. Ten more survivors (three guinea pigs, four mice, one rabbit, and two rats) were subjected to fewer periods of confinement (cf. Table I).

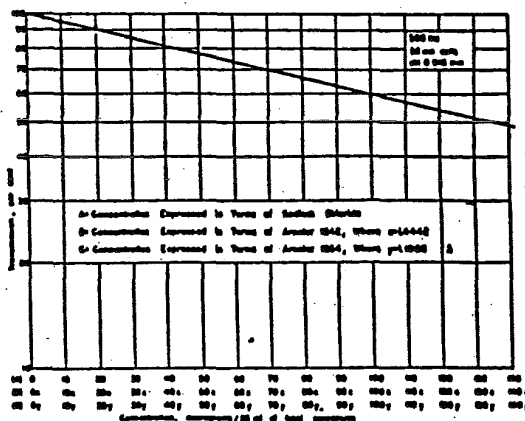


Fig. 4.
Standard curve for the determination of Aroclor 1242 and Aroclor 1254.

In another experiment (No. 3, Table I-A) in which animals were exposed to air bearing Aroclor 1242 in the concentration of 1.9 micrograms per liter (0.18 ppm) for seven hours per day on 150 days over a period of 214 days, the incidence of mortality among

TABLE I.
SUMMARY OF DATA ON MORTALITY AMONG ANIMALS EXPOSED TO THE VAPOR
OF AROCLOR 1242 OR AROCLOR 1254 IN AIR
(Higher concentrations)

Material	Concentration		Duration of Exposure (hours)	Number of Animals that Survived					Expt. No.
	γ/l	ppm		Cats	Guinea Pigs	Mice	Rabbits	Rats	
Aroclor 1242	6.83	0.66	17 x 7	1	6	10	4	10	1
Aroclor 1242	6.83	0.66	23 x 7	1	6 ¹	9 ^{1,2}	3 ¹	10 ^{1,2}	2
Aroclor 1254	5.40	0.51	23 x 7	1	6 ^{1,2}	10 ^{1,2}	4 ^{1,2}	10 ¹	1
Control	0	0	84 x 7	1	6 ^{1,2}	7 ¹	4 ¹	10 ^{1,2}	1

¹One of the animals was exposed on only 66 to 77 days

²One of the animals was exposed on only 41 to 55 days

³One of the animals was exposed on only 20 to 23 days

⁴Five of the mice were exposed on only 74 days

⁵Three of the mice were exposed on only 14 to 19 days

⁶One of the rabbits was exposed on only 19 days

TABLE I-A.
SUMMARY OF DATA ON MORTALITY AMONG ANIMALS EXPOSED TO THE VAPOR
OF AROCLOR 1242 OR AROCLOR 1254 IN AIR
(Lower concentrations)

Material	Concentration		Duration of Exposure (hours)	Number of Animals that Survived					Expt. No.
	γ/l	ppm		Cats	Guinea Pigs	Mice	Rabbits	Rats	
Aroclor 1242	1.90	0.18	150 x 7	1	6 ¹	8 ¹	3 ^{1,2}	10 ¹	2
Aroclor 1254	1.50	0.11	150 x 7	1	7 ^{1,2}	10 ^{1,2,3}	4 ^{1,2}	11 ¹	2
Control	0	0	150 x 7	1	6 ¹	10 ^{1,2,3,4,5}	3 ^{1,2}	10 ¹	2

¹One of the animals was exposed on only 26 to 27 days

²One of the animals was exposed on only 41 to 55 days

³One of the animals was exposed on only 130 to 131 days

⁴One of the animals was exposed on only 5 to 13 days

⁵One of the animals was exposed on only 107 to 120 days

⁶One mouse was exposed on only 64 days

the various species, with the exception of the rabbits (which died during an epidemic of pneumonia), was no greater than that encountered among a similarly constituted control group (Experiment No. 2, Table I-A).

One cat, five guinea pigs, six mice and nine rats survived throughout the entire period of their subjection to the vapor of Aroclor 1242. Seven additional survivors (one guinea pig, two mice, three rabbits, and one rat) were exposed intermittently to the vapor of Aroclor 1242 over a shorter period of time (cf. Table I-A). Among the group of controls (Experiment No. 2, Table I-A), one cat, five guinea pigs, four mice, two rabbits, and nine rats survived throughout the entire period of confinement, for seven hours on each of 150 days over 213 days in a chamber supplied with conditioned air. Eleven other control animals (one guinea pig, six mice, three rabbits, and one rat) survived during a shorter total period of intermittent confinement (Table I-A). No signs of intoxication were observed among experimental or control animals.

MORTALITY—AROCOLOR 1254: One cat, three guinea pigs, four mice, two rabbits, and nine rats survived throughout their exposure, for seven hours on each of 83 days over a period of 121 days, to air containing Aroclor 1254 in the concentration of 5.40 micrograms per liter (0.41 ppm). Certain other animals (three guinea pigs, six mice, two rabbits, and one rat) were killed for examination after 33 to 74 periods of exposure (Table I). The incidence of mortality from extraneous causes among the exposed and control animals (Experiment No. 1), with the exception of the rabbits, was comparable (Table I).

One cat, four guinea pigs, six mice, four rabbits, and 10 rats survived (Experiment 2, Table I-A) following their exposure for seven hours on each of 150 days over a period of 213 days to air containing Aroclor 1254 in the concentration of 1.5 micrograms per liter, (0.11 ppm). Eight other animals (three guinea pigs, four mice, and one rat) survived through 30 to 139 periods of exposure. The incidence of fatalities among the exposed group was slightly less than that encountered among the controls (Experiment 2, Table I-A).

TABLE II.
THE AVERAGE CHANGES IN WEIGHT OF EXPERIMENTAL AND CONTROL ANIMALS
(Higher concentrations)

Species of Animal	Number of Animals	Average Initial Weight (kg)	Average Change in Weight Expressed as Percentage of Initial Weight
Aroclor 1242 - 0.40 γ /l - Experiment No. 1			
Cat	1	2.803	+ 8.1
Guinea Pig	6	0.649	- 2.0
Mouse	10	0.036	+ 9.1
Rabbit	4	2.901	+ 7.7
Rat	10	0.328	+ 2.1
Aroclor 1242 - 0.83 γ /l - Experiment No. 2			
Cat	1	2.371	+22.2
Guinea Pig	6	0.404	+46.4
Mouse	9	0.021	+21.2
Rabbit	3	2.587	+50.5
Rat	10	0.212	+17.6
Aroclor 1254 - 0.40 γ /l - Experiment No. 1			
Cat	1	2.339	+22.1
Guinea Pig	6	0.409	+40.7
Mouse	10	0.021	+22.0
Rabbit	4	2.722	+39.7
Rat	10	0.227	+ 6.0
Conditioned Air - Controls - Experiment No. 1			
Cat	1	2.385	+ 6.6
Guinea Pig	6	0.418	+56.6
Mouse	7	0.022	+17.4
Rabbit	4	2.764	+44.5
Rat	10	0.211	+10.4

TABLE II-A.
THE AVERAGE CHANGES IN WEIGHT OF THE SURVIVORS AMONG THE ORIGINAL GROUPS
(Lower Concentrations)

Species of Animal	Number of Animals	Average Initial Weight (kg)	Average Change in Weight Expressed as Percentage of Initial Weight	P
Aroclor 1242 - 1.9 γ /l - Experiment No. 2				
Cat	1	1.799	+116.0	—
Guinea Pig	6	0.214	+ 43.1	0.05
Mouse	6	0.039	+ 31.5	0.05
Rat	9	0.214	+ 39.9	0.05
Aroclor 1254 - 1.5 γ /l - Experiment No. 2				
Cat	1	2.368	+ 18.2	—
Guinea Pig	4	0.487	+ 82.5	0.05
Mouse	6	0.0248	+ 17.6	0.05
Rabbit	4	2.988	+ 44.4	0.05
Rat	10	0.222	+ 22.7	0.05
Conditioned Air - Controls - Experiment No. 2				
Cat	1	2.329	- 7.5	—
Guinea Pig	6	0.509	+ 54.7	—
Mouse	6	0.0257	+ 12.2	—
Rabbit	4	2.622	+ 82.2	—
Rat	9	0.216	+ 22.7	—

¹Included two early replacements.

No general conclusion were noted among animals during GROWTH: The changes in various groups and II-A.

Although weighed during exposure, the guinea pig, despite a small increase in weight at the end of exposure.

The animals exposed to Aroclor 1242 in micrograms per liter or to Aroclor 1254, with the exception of those that were exposed to Aroclor 1254, grew equi-

Experiment 2. The growth of animals in the lower concentration of Aroclor 1242 (

SUMMARY OF THE BODY WEIGHT

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No general or specific signs of intoxication were noted among the experimental animals during or after their exposure.

GROWTH: The pertinent data relating to the changes in weight of the animals of the various groups are presented in Table II and II-A.

Although comparable controls were not weighed during the first experiment with Aroclor 1242 (17 x 7.0 hrs.—8.60 micrograms per liter) all of the animals, except the guinea pigs, appeared to gain normally in weight during the period of exposure. Despite a small net loss by the guinea pigs in this experiment, they were actually gaining weight at the termination of the period of exposure.

The animals exposed to the vapor of Aroclor 1242 in the concentration of 6.83 micrograms per liter (Experiment No. 2) or to Aroclor 1254 in the concentration of 5.40 micrograms per liter (Experiment No. 1), with the exception of the guinea pigs that were exposed to the vapor of Aroclor 1254, grew equally as well as the controls (Experiment No. 1, Table II).

The growth of the surviving experimental animals in the groups initially exposed to the lower concentrations of the vapor of Aroclor 1242 (Experiment No. 3, 1.9 micro-

grams per liter) and Aroclor 1254 (Experiment No. 2, 1.5 micrograms per liter) was unaffected. No significant differences in the average change in weight of the experimental and the control groups were found by the use of the "t" test (Table II-A).

WEIGHT OF LIVER AND KIDNEYS: The weights of the livers and kidneys and the relationships of their weights to the body weights (expressed as grams per 100 grams of body weight) of the animals that survived following exposure to the vapor of Aroclor 1242 in the concentration of 6.83 micrograms per liter, are shown in Table III, which also gives the corresponding data on the controls. Comparison by the "F" test of the variances of the ratios of the livers or kidneys to the body weights of rats, guinea pigs and rabbits exposed to the vapor of Aroclor 1242 (6.83 micrograms/liter), with those of the controls, revealed no significant differences ($P > 0.05$) except in the case of the livers of the rats. Application of the "t" test to the mean values (Table III) revealed no significant differences between the test and control groups of guinea pigs, rats, and rabbits.

Comparable values for rats, guinea pigs and rabbits exposed to Aroclor 1254 in the concentration of 5.40 micrograms per liter

TABLE III.
SUMMARY OF THE DATA ON THE RELATIONSHIP OF THE WEIGHT OF THE LIVER OR THE KIDNEYS TO THE BODY WEIGHT OF ANIMALS EXPOSED TO THE VAPOR OF AROCLOR 1242 OR AROCLOR 1254
Aroclor 1242 - 6.83 γ /l - 82 $\pm$ 7 hrs.
Aroclor 1254 - 5.40 γ /l - 83 $\pm$ 7 hrs.
Controls - 0 γ /l - 84 $\pm$ 7 hrs.

Compound	Organ	Species of Animal	n	Average Organ Weight (g)	Average Body Weight (g)	Ratio of Weight of Organ $\times$ 100 to Body Weight	t	P
Aroclor 1242	Liver	Rat	8	11.2	240	4.64	1.2521	0.20-0.30
Aroclor 1242	Liver	Guinea Pig	4	20.6	640	4.68	0.4591	> 0.50
Aroclor 1242	Liver	Rabbit	3	122.0	4,023	3.04	1.3026	0.10-0.20
Aroclor 1254	Liver	Rat	10	12.5	210	5.94	2.8692	0.01
Aroclor 1254	Liver	Guinea Pig	6	25.0	581	4.31	0.0000	0.50
Aroclor 1254	Liver	Rabbit	4	120.0	3,557	3.35	1.0122	0.50
Aroclor 1242	Kidneys	Rat	8	1.54	240	0.772	0.5542	0.50
Aroclor 1242	Kidneys	Guinea Pig	4	5.0	640	0.805	0.7257	0.10-0.20
Aroclor 1242	Kidneys	Rabbit	3	18.0	4,023	0.443	0.4177	0.50
Aroclor 1254	Kidneys	Rat	10	2.1	210	0.770	0.4190	0.50
Aroclor 1254	Kidneys	Guinea Pig	6	4.4	581	0.808	0.5411	0.50
Aroclor 1254	Kidneys	Rabbit	4	15.8	3,557	0.444	0.3163	0.50
Control	Liver	Rat	10	9.0	243	4.15	—	—
Control	Liver	Guinea Pig	6	30.3	364	4.51	—	—
Control	Liver	Rabbit	4	102.3	3,993	2.61	—	—
Control	Kidneys	Rat	10	2.0	243	0.807	—	—
Control	Kidneys	Guinea Pig	6	4.4	446	0.731	—	—
Control	Kidneys	Rabbit	4	19.5	3,993	0.504	—	—

are also given in Table III. Application of the "t" test to the differences in the mean values which characterized the experimental and control animals (Table III) shows that the weights of the livers of the exposed rats were significantly greater than those of the controls, the ratio of the liver to the body weight of the former being 5.34 g per 100 g of body weight, that of the latter being 4.15 g per 100 grams. In all other instances tested (livers and kidneys of both guinea pigs and rabbits and kidneys of rats) the differences were statistically insignificant.

The organs of the animals exposed to the lesser concentrations of the vapor of the Aroclors were not examined in this manner because of the borderline character of the results associated with the higher concentrations.

LIVER FUNCTION: Data were obtained as to the apparent prothrombin activity of the blood (measured by the method of Kato²) of certain animals that had been exposed (1) to the vapor of Aroclor 1242, in the con-

centration of 6.83 micrograms per liter; (2) to the vapor of Aroclor 1254 in the concentration of 5.40 micrograms per liter; and (3) to conditioned air alone. In Table IV the percentile relationships of the clotting power have been calculated arbitrarily by dividing 100 times the average clotting time of the blood of the control animals by the clotting time of the blood of the experimental animals on the same day. No diminution in the clotting power of the blood, as a measure of the impairment of the function of the liver, was induced by the exposure of the animals to the vapor of Aroclor 1242 in air in the concentration of 6.83 micrograms per liter, or to the vapor of Aroclor 1254 in the concentration of 5.40 micrograms per liter.

This functional test was not applied to animals subjected to the lower concentrations, because of the negative results obtained when the animals were subjected to the higher concentrations.

HEMATOLOGICAL RESULTS: Determina-

TABLE IV.
THE EFFECT OF EXPOSURE TO THE VAPOR OF AROCLOR 1242 OR AROCLOR 1254 IN AIR FOR SEVEN HOURS PER DAY ON FIVE DAYS PER WEEK OVER A PERIOD OF SEVERAL MONTHS UPON THE APPARENT PROTHROMBIN CONTENT OF THE BLOOD OF CATS AND RABBITS

Relation of "Prothrombin Time" of Control and Exposed Animals Expressed Arbitrarily in Terms of Percentage Change in Clotting Time of Blood 100 x Clotting Time of Blood of Control Animals		Time of Sampling in Relation to Period of Exposure	
Clotting Time of Blood of Exposed Animals		Days Before First Period	Serial Number of Period After Which Blood Was Drawn
Aroclor 1242 - 6.83 γ /l - Experiment No. 2			
Cat A-445	Rabbits (Average)		
100.0	99.6	1	
90.3	93.6	2	
118.6	105.1		10
101.5	99.0		30
97.4	98.0		45
118.3	103.7		59
	101.5		82
	100.0		1
Aroclor 1254 - 5.40 γ /l - Experiment No. 1			
Cat A-446	Rabbits (Average)		
100.0	97.1	1	
100.0	96.9	2	
	91.5		9
98.5	90.9		10
	99.6		15
113.6	102.6		30
	112.1		45
101.5	97.0		59
92.3	95.4		82
90.0	95.2		85
	100.0		1

¹Determination made 16 days after 31st period of exposure.

²Determination made 14 days after 31st period of exposure.

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TABLE V.
THE AVERAGE NUMBERS OF ERYTHROCYTES AND LEUCOCYTES AND THE AVERAGE CONCENTRATION OF HEMOGLOBIN IN THE PERIPHERAL BLOOD OF EXPERIMENTAL AND CONTROL ANIMALS

Compound	Concentration (γ/l)	Experiment Number	Species of Animal	Erythrocytes (Thousands) per mm ³	Leucocytes per mm ³	Hemoglobin g/100 ml
Aroclor 1242	6.83	2	Guinea Pig	5,761	10,112 ¹	14.1 ¹
			Rabbit	6,468	9,755	12.1
Aroclor 1254	5.40	1	Guinea Pig	3,844	12,927	14.5 ¹
			Rabbit	6,466 ²	10,511	12.2
Controls	0	1	Guinea Pig	5,755	12,134	12.9
			Rabbit	5,905	11,596	12.2

¹Value significantly less than that yielded by controls.
²Value significantly greater than that yielded by controls.

tions of the numbers of erythrocytes and leucocytes and of the hemoglobin content in the peripheral blood of the guinea pigs and rabbits subjected to inhalation of the vapor of Aroclor 1242 in the concentration of 6.83 micrograms per liter, are given in Table V, which also includes comparable results on control animals. Application of the "t" test to differences in the mean values for the experimental and control animals yielded borderline evidence of significant differences in the number of leucocytes and in the concentration of the hemoglobin in the blood of the guinea pigs. These differences were small and of opposite sign (the numbers of leucocytes in the blood of the test animals were low, while the hemoglobin content was high), as well as being subject to individual variations, and they cannot be regarded as of physiological significance. Somewhat comparable results were obtained when guinea pigs and rabbits were subjected to Aroclor 1254 in the concentration of 5.40 micrograms per liter. No physiological significance is attached to the slightly elevated hemoglobin content of the guinea pigs.

PATHOLOGICAL FINDINGS: In all of the experiments, animals were killed from one to 15 days after the final period of exposure.

In the first experiment with Aroclor 1242 (6.83 micrograms per liter), all of the animals were examined postmortem, and since gross examination of the viscera did not reveal any significant alterations, the tissues of only representative animals (one cat, four guinea pigs, six mice, four rabbits and six rats) were sectioned and examined microscopically. No abnormalities were found in the viscera of these animals.

In the second experiment with Aroclor 1242 (6.83 micrograms per liter), and in the

first experiment with Aroclor 1254 (5.40 micrograms per liter), which were carried out simultaneously, the deaths that occurred among the test and control animals were the apparent result of an appreciable incidence of pneumonia. The lesions of certain of the animals were those of frank pneumonia; in others such lesions were not fully developed. Degenerative changes in the viscera were usually found, in varying degrees of severity, in association with the pneumonia, but in certain animals, test and control alike, the degenerative changes were more evident than the pneumonia. The similarity of the lesions in test and control animals, and the lack of characteristic evidence of chemical pneumonitis, led to the reasonable, but not altogether certain, conclusion that all of these fatalities resulted from intercurrent disease among the animals, and not from the effects of their exposure to the Aroclors. For practical purposes, this conclusion was subjected to the critique of further experiments involving more prolonged exposure of animals to somewhat lower concentrations. Except in the case of the rats exposed to Aroclor 1254, the survivors subjected to the higher concentration of either Aroclor had normal viscera.

All of the animals exposed to the vapor of Aroclor 1242 in the concentration of 1.9 micrograms per liter were examined postmortem, and the viscera of most of them were examined microscopically. The few deaths among the exposed group were attributed to incidental infectious pulmonary disease. All exposed survivors, except two rabbits that had hepatic lesions of coccidiosis, had normal viscera. The control animals (Experiment No. 2) that died were found to have had pneumonia. Of those that sur-

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vived, one rat, two guinea pigs, and one mouse had focal or diffuse cytoplasmic vacuolation of the hepatic cells. The remaining control animals had normal viscera.

All of the animals exposed to the vapor of Aroclor 1254 in the concentration of 1.5 micrograms per liter were examined post-mortem, and the viscera of most of them were examined microscopically. A guinea pig that died exhibited chronic pyelonephritis, pulmonary hyperemia and edema, and degenerative lesions in the brain and liver. The visceral lesions were related, no doubt, to the renal infection. The deaths of four mice were attributable to acute bronchitis and pneumonia. The viscera of the cat that survived were normal. Of the seven guinea pigs that were living when the experiment was terminated, three had normal viscera and four had slight alterations of hepatic cells characterized by cytoplasmic vacuolation. Ten mice survived, and of these six had normal viscera and four had slight degenerative changes in the liver. Four rabbits killed one to 15 days after the last period of exposure had diffuse hepatic degeneration. The character of the lesions varied from cloudy to hyaline or hydropic degeneration and included varying degrees of fatty metamorphosis. The other viscera of these animals were normal. All of the rats were examined and found to have slightly to moderately severe degenerative lesions of the liver. The lesions of greatest severity were found in the rat that was killed and examined on the first day after the last period of exposure. Two rats had chronic pyelonephritis, and the remainder had slight degeneration of the renal tubules.

Discussion: In terms of mortality, growth (except that of guinea pigs) and non-occurrence of pathological changes, the vapor of Aroclor 1242 in the concentration of 8.6 micrograms per liter (approaching saturation) appeared to be non-injurious to experimental animals subjected thereto for seven hours on each of 17 days over a period of 24 days.

Experimental animals subjected to but a slightly lower concentration of Aroclor 1242 (6.83 micrograms per liter) for seven hours per day on each of 82 days over the period of 120 days suffered no injury on the basis of any of the following criteria: mortality, growth, pathology, organ enlarge-

ment, liver function or hematological changes.

More prolonged exposure of animals to a lower concentration of Aroclor 1242, (1.9 micrograms per liter) over the period of seven months was likewise without harm in terms of growth, mortality and the absence of pathological changes.

In view of this evidence it is suggested that the tentative allowable concentration of the vapor of Aroclor 1242 should be at least two micrograms per liter (2 mg per cu meter), which is twice that recommended by the American Conference of Governmental Industrial Hygienists² for a chlorinated diphenyl of unstated chlorine content.

The exposure of animals to the vapor of Aroclor 1254 in the concentration of either 5.40 or 1.5 micrograms per liter failed to induce harmful effects in the form of retardation of growth (except in the case of guinea pigs exposed to the higher concentration), or of mortality, but histopathologic evidence of apparently reversible hepatic cellular injury was found in the animals. These findings cannot certainly be attributed to the effects of Aroclor 1254 because of the appreciable incidence of pneumonia among both experimental and control animals. When these nonspecific toxic changes in the viscera of the animals were associated with pneumonia, they were readily explained thereby, but they were also found in animals that had been exposed to the vapor of Aroclor 1254 and were free of pneumonia. That these may have represented toxic effects of exposure to Aroclor 1254 finds support in the fact that the livers of the exposed rats (5.40 micrograms per liter) were significantly heavier in relation to their body weight, than were those of control rats. It would appear that this material, which is reported to contain 55% of chlorine, is somewhat more toxic than is Aroclor 1242, which contains only 42% of chlorine. Therefore it is suggested that the threshold concentration of 1 mg per cu meter of air recommended tentatively for safe industrial practice by the American Conference of Governmental Industrial Hygienists² is reasonable.

It should be noted that it was necessary to heat these Aroclors in order to increase the rate of volatilization sufficiently to attain the concentrations maintained in these

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experiments. To the extent that their industrial usage is carried out at ordinary temperatures, the hazard of their inhalation may well be slight or entirely absent.

Summary

PROLONGED intermittent exposure of animals to the vapor of Aroclor 1242 (1.90 to 8.63 micrograms per liter) demonstrated no injury. Prolonged exposure to comparable concentrations of Aroclor 1254 resulted in reversible degenerative changes in certain viscera.

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3. American Conference of Governmental Industrial Hygienists. Threshold Limits for 1955. *AMA Arch. Ind. Health*, 11:521, 1955.

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AFTER almost ten years of hard work on the part of a handful of interested and determined persons, the American Sanitary Engineering Inter-society Board has become a reality. The ASEIB is an incorporated organization sponsored by the American Society of Civil Engineers, the American Society for Engineering Education, the American Public Health Association, the American Water Works Association and the Federation of Sewage and Industrial Wastes Associations. The purpose of the Board is to improve the practice, elevate the standards, and advance the cause of sanitary engineering. The term "sanitary engineering" is employed in the broad sense as defined by the National Research Council, and embraces the entire concept of engineering biotechnology application for control of environmental stresses. As most readers know, this definition includes industrial hygiene, air pollution control and radiological health. The Board of Trustees of the ASEIB is made up of three representatives from each of the sponsoring bodies and three elected at large by the Board. We are happy to announce that two of the Trustees at large are industrial hygiene engineers, namely LT. COL. ALVIN F. MEYER, JR., Headquarters Strategic Air Command, and ALLEN D. BRANDT, Bethlehem Steel Company.

One of the most important functions of the ASEIB will be examining qualified licensed professional engineers and granting certificates of special knowledge in sanitary engineering or one of its specialties to those who show acceptable competency in the examination. In this respect the Board will function in a manner not unlike that of the American Boards of medicine. For an initial period, outstanding members of the profession having not less than 15 years acceptable experience will be granted certificates without examination. Persons who have been certified by the board will automatically become members of the American Academy of Sanitary Engineers which is the register of holders of such certificates.

The board was incorporated in October, 1955. At the present time the Chairman of the Board is PROFESSOR EARNEST BOYCE, University of Michigan; Vice-Chairman is W. A. HARDENBERG; Treasurer is R. S. RANKIN; Secretary is FRANCIS S. ELDER. It is contemplated that applications for certification will become available in June. All qualified industrial hygiene engineers, air pollution control engineers and radiation safety engineers having not less than eight years experience in this work are urged to apply for certification. The benefits that will result from this movement and the advantages that will accrue to all who become certified are many. What is more, they will increase with the passing years. Application forms may be obtained from the Secretary, FRANCIS S. ELDER, 33 West 39th Street, New York 18, New York, or from members of the Board of Trustees.

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PROJECT NO. 4-163-12
REPORT FILE

YOUNGER LABORATORIES

Biochemists ... Pharmacologists ... Analysts

128 CLIFF CAVE ROAD
ST. LOUIS 29, MISSOURI
PHONE: TILDEN 6-2540

Certificate of Analysis

March 4th, 1963

SUBJECT -

At ...

Toxicological Investigation Of: Inerteen PPO

Monsanto Sample Number 22

Monsanto Project Number Y-63-12

STUDY CONDUCTED FOR -

Monsanto Chemical Company, St. Louis, Missouri

EXPERIMENTAL PROCEDURE -

A) Oral LD₅₀ (Rats)

The undiluted compound was fed by stomach tube to Sprague-Dawley strain albino rats.

After the approximate Minimum Lethal Dose was determined, groups of male and female rats were fed in increasing doses at increments of 0.1 fractional log intervals at four levels designed to blanket the toxicity range thereby supplying data for calculation of the LD₅₀ which was done according to a modification of the method of E. J. de Beer.

Observations were made for toxic symptoms and the viscera of the animals that succumbed were examined macroscopically.

The data are shown in Table I.

B) Skin Absorption MLD (Rabbits)

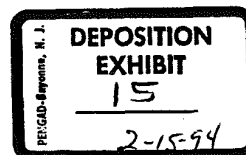
The undiluted compound was applied in increasing doses at increments of 0.2 fractional log intervals to the closely clipped, intact skin of New Zealand white female rabbits.

The treated areas were covered with plastic strips and the animals placed in wooden stocks for periods up to twenty-four hours, after which time they were assigned to individual cages.

Observations were made for toxic symptoms and the viscera of the animals that succumbed were examined macroscopically.

The data are shown in Table II.

NEV 017318



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To: Monsanto Chemical Company
 St. Louis, Missouri
 Younger Laboratories Certificate of Analysis - Page 2 (3/4/63) - Y-63-12

EXPERIMENTAL PROCEDURE - (Continued)

C) Skin Irritation (Rabbits)

The undiluted compound was applied to the clipped, intact skin of albino rabbits and removed after twenty-four hours. The application was covered with plastic strips to retard evaporation.

Observations were made over a period of several days for irritation.

The data, scored according to the method of Draize, Woodard and Calvery (Journal of Pharm. and Exp. Therapeutics, Volume 82, December, 1944) are shown in Table III.

D) Eye Irritation (Rabbits)

0.1 Milliliter of undiluted sample was placed in the conjunctival sac of the right eye of each of three albino rabbits and observations made over a period of several days for inflammation.

The eyes were rinsed with warm isotonic saline solution after twenty-four hours.

The data, scored according to the method of Draize, et al, are shown in Table IV.

E) Vapor Inhalation (Rats)

Four 150-gram male rats were placed in a glass desiccator, 250 mm in diameter, and exposed for six hours to a concentrated atmosphere of vapors produced by passing a stream of air through 100 milliliters of the compound contained in a 250-milliliter tall form gas washing bottle with fritted disc. Vapors from the bottle passed into a one liter bottle to remove droplets and then into the chamber. Air flow through the sample was 6 liters per minute as measured by a calibrated rotameter. This was sufficient to violently agitate the liquid. No supplementary air was introduced inasmuch as the above supply was ample for the animals oxygen requirements.

The animals were observed for behavior and, since there were no deaths, all were held for ten days observation.

The data are shown in Table V.

SUMMARY -

Inerteen PPO

A) Oral LD₅₀ (Rats)

The Oral LD₅₀ for male and female rats was placed at 2370 milligrams per kilogram with lower and upper limits of 2095 to 2690 milligrams per kilogram.

The compound was classed as slightly toxic by oral ingestion in male and female rats.

B) Skin Absorption MLD (Rabbits)

The Minimum Lethal Dose by Skin Absorption in female rabbits was found to be greater than 3160 milligrams per kilogram and less than 5010 milligrams per kilogram.

The compound was classed as slightly toxic by skin absorption in female rabbits.

NEV 017319

P3

To: Monsanto Chemical Company
St. Louis, Missouri
Younger Laboratories Certificate of Analysis - Page 3 (3/4/63) - Y-63-12

SUMMARY - (Continued)

C) Skin Irritation (Rabbits)

The compound was classed as a moderate skin irritant.

The average maximum score was 4.6 out of a possible 8 in twenty-four hours.

D) Eye Irritation (Rabbits)

The compound was classed as a moderate eye irritant.

The average maximum score was 35.6 out of a possible 110 in one hour.

E) Vapor Inhalation (Rats)

All animals survived the six hour exposure as well as the following ten day observation period.

It was concluded that the vapors were slightly toxic under conditions of the test.

YOUNGER LABORATORIES

Fred M. Younger
BY: FRED M. YOUNGER

The material in this report is to be used in development of the product and may be given to responsible sales contacts, but it is not to be used by them in advertising copy. The source of this material is not to be divulged until it appears in formal publications. No exceptions to the established rule may be made without the approval of the Medical Department in St. Louis. Customers' inquiries regarding matters of toxicity are to be referred as before to the Medical Department in St. Louis for reply.

— Monsanto Chemical Company

NEV 017320

To: Monsanto Chemical Company
St. Louis, Missouri

Younger Laboratories Certificate of Analysis - Page 4 (3/4/63) - Y-63-12

TABLE I

THE ORAL LD₅₀ OF 'Inerteen PPO' FOR RATS

Sample Fed Undiluted

Animal No. - Sex	Weight Gm.	Dose Mg./Kg.	Fate
1- Female	220	1580	Survived
2- Female	210	1580	Survived
3- Male	235	1580	Survived
4- Male	240	1580	Survived
5- Female	215	1580	Survived
6- Female	225	2000	Survived
7- Male	245	2000	Survived
8- Male	240	2000	Died
9- Female	210	2000	Died
10- Female	220	2000	Survived
11- Male	235	2510	Died
12- Male	245	2510	Survived
13- Female	205	2510	Died
14- Female	215	2510	Died
15- Male	250	2510	Survived
16- Male	235	3160	Died
17- Female	210	3160	Died
18- Female	220	3160	Died
19- Male	235	3160	Survived
20- Male	245	3160	Died

DISCUSSION -

The Oral LD₅₀ for male and female rats was placed at 2370 milligrams per kilogram with lower and upper limits of 2095 to 2690 milligrams per kilogram.

The compound was classed as slightly toxic by oral ingestion in male and female rats.

Survival time was approximately twenty-four hours to six days with most deaths occurring in three to five days.

Toxic symptoms included severe diarrhea, loss of appetite, increasing weakness, and collapse.

At autopsy there was marked liver and renal hyperemia as well as pulmonary congestion.

NEV 017321

To: Monsanto Chemical Company
St. Louis, Missouri
Younger Laboratories Certificate of Analysis - Page 5 (3/4/63) - Y-63-12

T A B L E II

THE MINIMUM LETHAL DOSE OF 'Inerteen PPO'
BY SKIN ABSORPTION IN RABBITS

Sample Applied Undiluted

Animal No. - Sex	Weight	Dose	Weight Change	Fate
	Kg.	Mg./Kg.	5 Days Later Kg.	
1 - Female	2.3	794	+ 0.2	Survived
2 - Female	2.1	1260	0.0	Survived
3 - Female	2.4	2000	0.0	Survived
4 - Female	2.3	3160	- 0.1	Survived
5 - Female	2.5	5010	- 0.4	Died -- 5 Days
6 - Female	2.7	7940	- 0.6	Died -- 6 Days

DISCUSSION -

The Minimum Lethal Dose by Skin Absorption in female rabbits was found to be greater than 3160 milligrams per kilogram and less than 5010 milligrams per kilogram.

The compound was classed as slightly toxic by skin absorption in female rabbits.

Survival time was five to six days.

The animals that succumbed lost appetite and wasted away. Movement reduced as the animals weakened but no definite paralytic symptoms were observed.

At autopsy the liver had a jaundiced-like appearance and there was pulmonary hyperemia macroscopically.

NEV 017322

To: Monsanto Chemical Company
St. Louis, Missouri
Younger Laboratories Certificate of Analysis - Page 6 (3/4/63) - Y-63-12

TABLE III

SKIN IRRITATION IN RABBITS AFTER APPLICATION OF 'Inerteen PPO'

Sample Applied Undiluted

<u>Animal Number</u>	<u>Numerical Evaluation At The End Of</u>					
	<u>1 Hour</u>	<u>24 Hours</u>	<u>48 Hours</u>	<u>72 Hours</u>	<u>120 Hours</u>	<u>168 Hours</u>
1	2	4	4	3	2	1
2	3	5	4	2	2	1
3	3	5	5	4	2	1
Average	2.6	4.6	4.3	3.0	2.0	1.0

DISCUSSION -

The compound was classed as a moderate skin irritant.

The average maximum score was 4.6 out of a possible 8 in twenty-four hours.

After one hour there was well-defined erythema with barely perceptible swelling for an average score of 2.6 out of a possible 8. Inflammation increased overnight at which time the average score was 4.6 based upon moderate redness and slight edema. Daily improvement was noted after the application was removed. Irritation reduced to barely perceptible erythema on all animals within seven days.

NEV 017323

To: Monsanto Chemical Company
St. Louis, Missouri

Younger Laboratories Certificate of Analysis - Page 7 (3/4/63) - Y-63-12

T A B L E IV

EYE IRRITATION IN RABBITS AFTER APPLICATION OF 'Inerteen PPO'

Sample (0.1 Milliliter) Applied Undiluted

<u>Animal Number</u>	<u>Numerical Evaluation At The End Of</u>					
	<u>1 Hour</u>	<u>24 Hours</u>	<u>48 Hours</u>	<u>72 Hours</u>	<u>120 Hours</u>	<u>168 Hours</u>
1	37	30	21	15	8	4
2	29	21	15	9	4	0
3	41	30	19	12	6	0
Average	35.6	27.0	18.3	12.0	6.0	1.3

DISCUSSION -

The compound was classed as a moderate eye irritant.

The average maximum score was 35.6 out of a possible 110 in one hour.

Moderate discomfort was shown following application.

Within one hour there was mild edema, copious discharge, mild to moderate redness of the conjunctivae, and slight iris congestion and corneal dullness for an average score of 35.6 out of a possible 110. Discharge and cloudiness decreased overnight. Iris clarity was normal within five days and within seven days two of three animals were free of inflammation.

NEV 017324

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To: Monsanto Chemical Company
St. Louis, Missouri
Younger Laboratories Certificate of Analysis - Page 8 (3/4/63) - Y-63-12

T A B L E V

INHALATION OF 'Inerteen PPO' VAPORS BY RATS

Average Temperature Inside Chamber 76° F.
Average Relative Humidity Inside Chamber 47 %

<u>Animal No. - Sex</u>	<u>Fate</u>	<u>Observations During Exposure</u>
1 - Male	Survived	Mild eye and nasal irritation after twenty to thirty minutes ...
2 - Male	Survived	Moderate discomfort ...
3 - Male	Survived	Lethargy ...
4 - Male	Survived	Occasional moderate dyspnea ... No serious weakness ... Slight nasal discharge.

DISCUSSION -

All animals survived the six hour exposure as well as the following ten day observation period.

It was concluded that the vapors were slightly toxic under conditions of the test.

Mild inflammation of the nasal mucosa was evident when the animals were removed from the chamber. Breathing was slightly irregular but no rasping sound was detected and the animals were only mildly weakened. Animal behavior was practically normal, in forty-eight hours. No respiratory complications developed during the ten day observation period.

NEV 017325

PROJECT NO. Y-42-11
REPORT FILE

YOUNGER LABORATORIES

Biochemists ... Pharmacologists ... Analysts

128 CLIFF CAVE ROAD
ST. LOUIS 29, MISSOURI

PHONE: TILDEN 6-2540

Certificate of Analysis

March 4th, 1963

SUBJECT -

Toxicological Investigation Of: Pyranol 1470

Monsanto Sample Number 21

Monsanto Project Number Y-63-11

STUDY CONDUCTED FOR -

Monsanto Chemical Company, St. Louis, Missouri

EXPERIMENTAL PROCEDURE -

A) Oral LD₅₀ (Rats)

The undiluted compound was fed by stomach tube to Sprague-Dawley strain albino rats.

After the approximate Minimum Lethal Dose was determined, groups of male and female rats were fed in increasing doses at increments of 0.1 fractional log intervals at four levels designed to blanket the toxicity range thereby supplying data for calculation of the LD₅₀ which was done according to a modification of the method of E. J. de Beer.

Observations were made for toxic symptoms and the viscera of the animals that succumbed were examined macroscopically.

The data are shown in Table I.

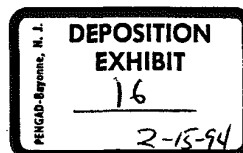
B) Skin Absorption MLD (Rabbits)

The undiluted compound was applied in increasing doses at increments of 0.2 fractional log intervals to the closely clipped, intact skin of New Zealand white female rabbits.

The treated areas were covered with plastic strips and the animals placed in wooden stocks for periods up to twenty-four hours, after which time they were assigned to individual cages.

Observations were made for toxic symptoms and the viscera of the animals that succumbed were examined macroscopically.

The data are shown in Table II.



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To: Monsanto Chemical Company
St. Louis, Missouri

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EXPERIMENTAL PROCEDURE - (Continued)

C) Skin Irritation (Rabbits)

The undiluted compound was applied to the clipped, intact skin of albino rabbits and removed after twenty-four hours. The application was covered with plastic strips to retard evaporation.

Observations were made over a period of several days for irritation.

The data, scored according to the method of Draize, Woodard and Calvery (Journal of Pharm. and Exp. Therapeutics, Volume 82, December, 1944) are shown in Table III.

D) Eye Irritation (Rabbits)

0.1 Milliliter of undiluted sample was placed in the conjunctival sac of the right eye of each of three albino rabbits and observations made over a period of several days for inflammation.

The eyes were rinsed with warm isotonic saline solution after twenty-four hours.

The data, scored according to the method of Draize, et al, are shown in Table IV.

E) Vapor Inhalation (Rats)

Four 150-gram male rats were placed in a glass desiccator, 250 mm in diameter, and exposed for six hours to a concentrated atmosphere of vapors produced by passing a stream of air through 100 milliliters of the compound contained in a 250-milliliter tall form gas washing bottle with fritted disc. Vapors from the bottle passed into a one liter bottle to remove droplets and then into the chamber. Air flow through the sample was 6 liters per minute as measured by a calibrated rotameter. This was sufficient to violently agitate the liquid. No supplementary air was introduced inasmuch as the above supply was ample for the animals oxygen requirements.

The animals were observed for behavior and, since there were no deaths, all were held for ten days observation.

The data are shown in Table V.

SUMMARY -

Pyranol 1470

A) Oral LD₅₀ (Rats)

The Oral LD₅₀ for male and female rats was placed at 2250 milligrams per kilogram with lower and upper limits of 1990 to 2555 milligrams per kilogram.

The compound was classed as slightly toxic by oral ingestion in male and female rats.

B) Skin Absorption MLD (Rabbits)

The Minimum Lethal Dose by Skin Absorption in female rabbits was found to be greater than 2000 milligrams per kilogram and less than 3160 milligrams per kilogram. Animal #3, dosed at 2000 milligrams per kilogram, very nearly succumbed.

The compound was classed as slightly toxic by skin absorption in female rabbits.

NEV 105132

80

To: Monsanto Chemical Company
St. Louis, Missouri
Younger Laboratories Certificate of Analysis - Page 3 (3/4/63) - Y-63-11

SUMMARY - (Continued)

C) Skin Irritation (Rabbits)

The compound was classed as a moderate skin irritant.

The average maximum score was 4.6 out of a possible 8 in twenty-four hours.

D) Eye Irritation (Rabbits)

The compound was classed as a moderate eye irritant.

The average maximum score was 32.6 out of a possible 110 in one hour.

E) Vapor Inhalation (Rats)

All animals survived the six hour exposure as well as the following ten day observation period.

It was concluded that the vapors were slightly toxic under conditions of the test.

YOUNGER LABORATORIES

Fred M. Younger
BY: FRED M. YOUNGER

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— Monsanto Chemical Company

NEV 105133

To: Monsanto Chemical Company
St. Louis, Missouri

Younger Laboratories Certificate of Analysis - Page 4 (3/4/63) - Y-63-11

TABLE I

THE ORAL LD₅₀ OF 'Pyranol 1470' FOR RATS

Sample Fed Undiluted

Animal No. - Sex	Weight	Dose	Fate
	Gm.	Mg. / Kg.	
1- Female	225	1580	Survived
2- Female	210	1580	Survived
3- Male	235	1580	Survived
4- Male	250	1580	Survived
5- Female	215	1580	Survived
6- Female	220	2000	Survived
7- Male	240	2000	Survived
8- Male	245	2000	Survived
9- Female	230	2000	Died
10- Female	215	2000	Survived
11- Male	235	2510	Died
12- Male	240	2510	Survived
13- Female	220	2510	Died
14- Female	210	2510	Died
15- Male	235	2510	Died
16- Male	225	3160	Died
17- Female	220	3160	Died
18- Female	215	3160	Died
19- Male	235	3160	Died
20- Male	245	3160	Died

DISCUSSION -

The Oral LD₅₀ for male and female rats was placed at 2250 milligrams per kilogram with lower and upper limits of 1990 to 2555 milligrams per kilogram.

The compound was classed as slightly toxic by oral ingestion in male and female rats.

Survival time was approximately twenty-four hours to three days with most deaths occurring in two to three days.

Toxic symptoms included increasing weakness, severe diarrhea, tremors, and collapse.

At autopsy there was inflammation of the gastric mucosa, liver discoloration, and renal hyperemia macroscopically.

NEV 105134

To: Monsanto Chemical Company
 St. Louis, Missouri
 Younger Laboratories Certificate of Analysis - Page 5 (3/4/63) - Y-63-11

T A B L E II
 THE MINIMUM LETHAL DOSE OF 'Pyranol 1470'
 BY SKIN ABSORPTION IN RABBITS

Sample Applied Undiluted

Animal No. - Sex	Weight Kg.	Dose Mg./Kg.	Weight Change	Fate
			5 Days Later Kg.	
1 - Female	2.5	794	- 0.1	Survived
2 - Female	2.2	1260	- 0.1	Survived
3 - Female	2.3	2000	- 0.2	Survived
4 - Female	2.6	3160	- 0.3	Died - 10 Days
5 - Female	2.5	5010	- 0.2	Died - 7 Days
6 - Female	2.5	7940	- 0.4	Died - 7 Days

DISCUSSION -

The Minimum Lethal Dose by Skin Absorption in female rabbits was found to be greater than 2000 milligrams per kilogram and less than 3160 milligrams per kilogram. Animal #3, dosed at 2000 milligrams per kilogram, very nearly succumbed.

The compound was classed as slightly toxic by skin absorption in female rabbits.

Survival time was seven to ten days.

Toxic symptoms included loss of appetite, lethargy, and increasing weakness. Weight losses ran to forty per cent of starting weight.

At autopsy there was liver discoloration and renal hyperemia macroscopically.

NEV 105135

To: Monsanto Chemical Company
St. Louis, Missouri
Younger Laboratories Certificate of Analysis - Page 6 (3/4/63) - Y-63-11

TABLE III

SKIN IRRITATION IN RABBITS AFTER APPLICATION OF 'Pyranol 1470'

Sample Applied Undiluted

Animal Number	Numerical Evaluation At The End Of					
	1 Hour	24 Hours	48 Hours	72 Hours	120 Hours	168 Hours
1	3	5	4	3	2	1
2	2	4	3	2	1	0
3	3	5	4	2	2	1
Average	2.6	4.6	3.6	2.3	1.6	0.6

DISCUSSION -

The compound was classed as a moderate skin irritant.

The average maximum score was 4.6 out of a possible 8 in twenty-four hours.

Well-defined redness with very slight edema developed within an hour for an average score of 2.6 out of a possible 8. Overnight there was well-defined to moderate erythema and slight edema increasing the average score to 4.6. No blistering was observed. Inflammation gradually receded following removal of the application. After seven days one animal was given a score of zero but very slight redness was still present on the remaining animals.

NEV 105136

To: Monsanto Chemical Company
St. Louis, Missouri
Younger Laboratories Certificate of Analysis - Page 7 (3/4/63) - Y-63-11

T A B L E IV

EYE IRRITATION IN RABBITS AFTER APPLICATION OF 'Pyranol 1470'

Sample (0.1 Milliliter) Applied Undiluted

Animal Number	Numerical Evaluation At The End Of					
	1 Hour	24 Hours	48 Hours	72 Hours	120 Hours	168 Hours
1	35	26	17	12	6	0
2	28	21	13	8	4	0
3	35	30	20	12	6	0
Average	32.6	25.6	16.6	10.6	5.3	0.0

DISCUSSION -

The compound was classed as a moderate eye irritant.

The average maximum score was 32.6 out of a possible 110 in one hour.

There was intermittent pawing at the eyes for several minutes following application.

After one hour the average score was 32.6 out of a possible 110. Slight edema, slight to moderate redness of the conjunctivae, copious discharge, and slight iris congestion developed. The corneal area cleared somewhat in twenty-four hours and within five days iris and cornea were normal. Conjunctivitis and edema cleared completely within seven days.

NEV 105137

85

To: Monsanto Chemical Co., Inc.
St. Louis, Missouri
Younger Laboratories Certificate of Analysis - Page 8 (3/4/63) - Y-63-11

T A B L E V

INHALATION OF 'Pyranol 1470' VAPORS BY RATS

Average Temperature Inside Chamber 78° F.
Average Relative Humidity Inside Chamber 42 %

<u>Animal No. - Sex</u>	<u>Fate</u>	<u>Observations During Exposure</u>
1 - Male	Survived	Occasional pawing at the eyes in ten to fifteen minutes ...
2 - Male	Survived	
3 - Male	Survived	Slight nasal discharge ...
4 - Male	Survived	Some discomfort ...
		No real weakness ...
		Occasional irregular breathing.

DISCUSSION -

All animals survived the six hour exposure as well as the following ten day observation period.

It was concluded that the vapors were slightly toxic under conditions of the test.

The vapors produced mild nasal and eye inflammation as well as some discomfort. No weakness of consequence was noted at the end of exposure and no bronchial rales developed.

Activity and appetite returned to normal within twenty-four hours. Nasal inflammation disappeared within seventy-two hours. No respiratory complications developed during the ten day observation period.

NEV 105138

Project 1254
PROJECT NO. BTL-62-4
REPORT FILE

Industrial **BIO-TEST Laboratories, Inc.**
1810 FRONTAGE ROAD
NORTHBROOK, ILLINOIS
Telephone CLashwood 2-3030

REPORT TO
MONSANTO CHEMICAL COMPANY
SUBACUTE DERMAL TOXICITY OF
AROCLOL 1254 CP 98365

BTL-62-4
3-25-63

DEPOSITION
EXHIBIT
17
2-15-94
FENGLE-Bayonne, N. J.

NEV 009120

REPORT TO
MONSANTO CHEMICAL COMPANY
SUBACUTE DERMAL TOXICITY OF
AROCLOR 1254

I. Introduction and Outline of Procedure

A sample identified as Aroclor 1254 was received from Monsanto Chemical Company for evaluation of 20-day subacute dermal toxicity in albino rabbits.

Skin applications of the test material, in the form of a corn oil solution, were made daily to four test groups consisting of two rabbits each (one male and one female) for a period of 20 days. These test groups corresponded to daily dermal doses of 10, 50, 100 and 200 mg/kg/day respectively.

In addition to the four test groups, a treated control group of two rabbits was also employed. The animals in this group received daily dermal applications of corn oil comparable to the volume administered to the highest test dose group.

The rabbits in all groups were housed individually over the period of investigation and observations were made with respect to body weight effects, incidence of mortality, reactions displayed and status of the

NEV 009121

hematopoietic and urinary systems. Special emphasis was placed on observations for skin irritation produced by the test material.

Following the 20-day application period, the rabbits were kept under observation until an additional 14 days had elapsed. At the end of this period, final mortality tabulations were made and all surviving rabbits from each test and control group were sacrificed for pathologic studies. Gross autopsies were performed and a complete set of representative tissues and organs was taken from each rabbit and preserved in formalin. Microscopic examinations of selected tissues and organs were made to ascertain the presence or absence of histopathologic change as a result of dermal applications of the test material at the dose levels employed.

The dosage schedule and other pertinent data are presented in Table I.

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— Monsanto Chemical Company

NEV 009122

TABLE I
20-Day Subacute Dermal Toxicity - Albino Rabbits
Dosage Schedule

Group	Number of Animals	Dose* (mg/kg/day)	Number of Scheduled Consecutive Applications
T-C	2	**	20
T-I	2	10	20
T-II	2	50	20
T-III	2	100	20
T-IV	2	200	20

* Administered as a 4.0 per cent solution in corn oil.

** The treated control rabbits received doses of corn oil comparable in volume to that applied to the rabbits of the high dose group.

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II. Investigational Procedure

A. Experimental Animals

The animals employed in the 20-day subacute dermal toxicity study were adult, New Zealand strain albino rabbits. The body weights of the test animals were in the two-to-three kilogram range. A total of 10 adult rabbits were selected from a larger population after examination of each animal for general physical well-being. Each animal selected for the experiment was housed individually in a wire-bottomed stainless steel rabbit cage for the duration of the test. All rabbits were fed a standard laboratory rabbit ration* plus water ad libitum.

B. Method of Application

In preparation for the test, the back of each rabbit was shaved using electric clippers. The exposure site thus prepared on each animal constituted approximately ten per cent of the total body surface area. The animals were then returned to their stock cages, and 24 hours were allowed to elapse before the first percutaneous applications were made. This period permitted the skin to recover from the slight disturbance of the stratum corneum caused by the clipping procedure and also permitted the healing of any microscopic abrasions possibly produced during the shaving procedure.

At the end of the 74-hour recovery period, the calculated doses of the test material in the form of a 4.0 per cent (w/v) corn oil solution

* Rockland Rabbit Ration, Rockland Farms, New City, New York.

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were gently distributed (without rubbing) over the entire application sites on the test rabbits.

The animals in the treated control group were treated in the same manner as those in the test group with the exception that corn oil alone was applied to the skin.

Following the dermal applications, the animals were returned to their individual cages to await testing on the following day.

The above schedule was followed every day, including weekends, for a total of 20 consecutive days.

C. Body Weight Effects

On the morning of the first test day, the body weight of each rabbit in every group was determined and recorded. Thereafter, individual weighings were made weekly and the data recorded as an index to body weight effects. Dose calculations were made weekly to coincide with changes in body weight.

D. Mortality and Reactions

Checks for mortality and abnormal behavioral reactions were made daily during the 20-day test period as well as during the 14-day observation period. Special emphasis was placed on observations for local skin reactions in order to determine the degree of skin irritation produced by the test material.

E. Hematologic Studies and Urine Analyses

Blood studies including determinations of hemoglobin concentration, erythrocyte count, and both total and differential leukocyte

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counts, were made at the beginning of the study and at the end of the 20-day test period. Urine analyses for reducing substances and microscopic elements were also done at these time intervals. The blood studies and urine analyses were done on the individual rabbits in each group.

F. Gross and Microscopic Pathologic Studies

Arrangements were made to subject any animal which might die during the test to a gross autopsy. Also, in those instances where post-mortem changes were not advanced, sections of representative tissues and organs were scheduled to be taken for histopathologic study.

At the conclusion of the investigational period, all surviving rabbits in each group were sacrificed and gross and microscopic studies were performed. The following tissues and organs were included in the microscopic studies: heart, aorta, lung, trachea, liver, gall bladder, pancreas, esophagus, stomach, small intestine, caecum, colon, spleen, cervical and mesenteric lymph nodes, kidney, urinary bladder, testis, ovary, prostate, uterus, pituitary, adrenal gland, submaxillary salivary gland, thyroid, parathyroid, skeletal muscle, bone marrow, peripheral nerve, spinal cord, brain, and skin.

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III. Results

A. Body Weight Data

Weekly individual body weights for each test group and the control group are assembled in Table II.

TABLE II
Body Weight Data

Group	Animal Number and Sex	Individual Body Weights (Kilograms)				Observation Day Number 14
		0	7	14	20	
T-C	1-M	2.31	2.35	2.49	2.57	2.74
	2-F	2.50	2.63	2.68	2.80	2.96
T-I	1-M	2.41	2.53	2.60	2.69	2.82
	2-F	2.72	2.85	2.89	2.95	3.16
T-II	1-M	2.10	1.70	(1.36)	-	-
	2-F	2.16	1.96	1.96	2.04	2.18
T-III	1-M	2.55	2.35	2.38	2.18	2.04
	2-F	2.72	2.80	2.26	1.84	(1.80)
T-IV	1-M	2.61	(2.01)	-	-	-
	2-F	2.53	(1.87)	-	-	-

Note: Figures in parentheses represent autopsy weights.

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B. Mortality

The mortality data are summarized in Table III. The data indicate the number dead and the total number of doses received prior to death. The female of Group III died on the second day of the 14-day observation period.

The mortality data were statistically analyzed employing the technique of Litchfield and Wilcoxon*, and the mean subacute percutaneous lethal dose (LD_{50}) was calculated together with the $LD_{0.01}$ and the $LD_{99.99}$. For the purpose of these calculations, the test period was assumed to be 34 days, i.e. a 20-day application period plus a two-week observation period. All animals that died during the interval, at each dose level, were used in establishing the per cent mortality for the dose level. The data are presented in Table IV and are graphically illustrated in Figure 1.

* J. T. Litchfield, Jr. and F. Wilcoxon, "A Simplified Method of Evaluating Dose-Effect Experiments", J. Pharm. & Exp. Ther., Vol. 96, No. 2; pp. 99-113 (1949).

TABLE III
Summary of Mortality Data

Group	Number Tested	Number Dead	Sex	Number of Consecutive Doses Received Prior to Death
T-C	2	0	-	-
T-I	2	0	-	-
T-II	2	1	F	13
T-III	2	1	M	20
T-IV	2	1	M	5
		1	F	6

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TABLE IV

Statistical Analysis of Mortality Data

Dose mg/kg/day	Number Dead	Number Tested	Observed Per Cent Dead	Expected Per Cent Dead
10	0	2	0	0.2
50	1	2	50	26.5
100	1	2	50	66.0
200	2	2	100	92.5

Mean Subacute Dermal Lethal Dose (LD₅₀) = 75 mg/kg/day*

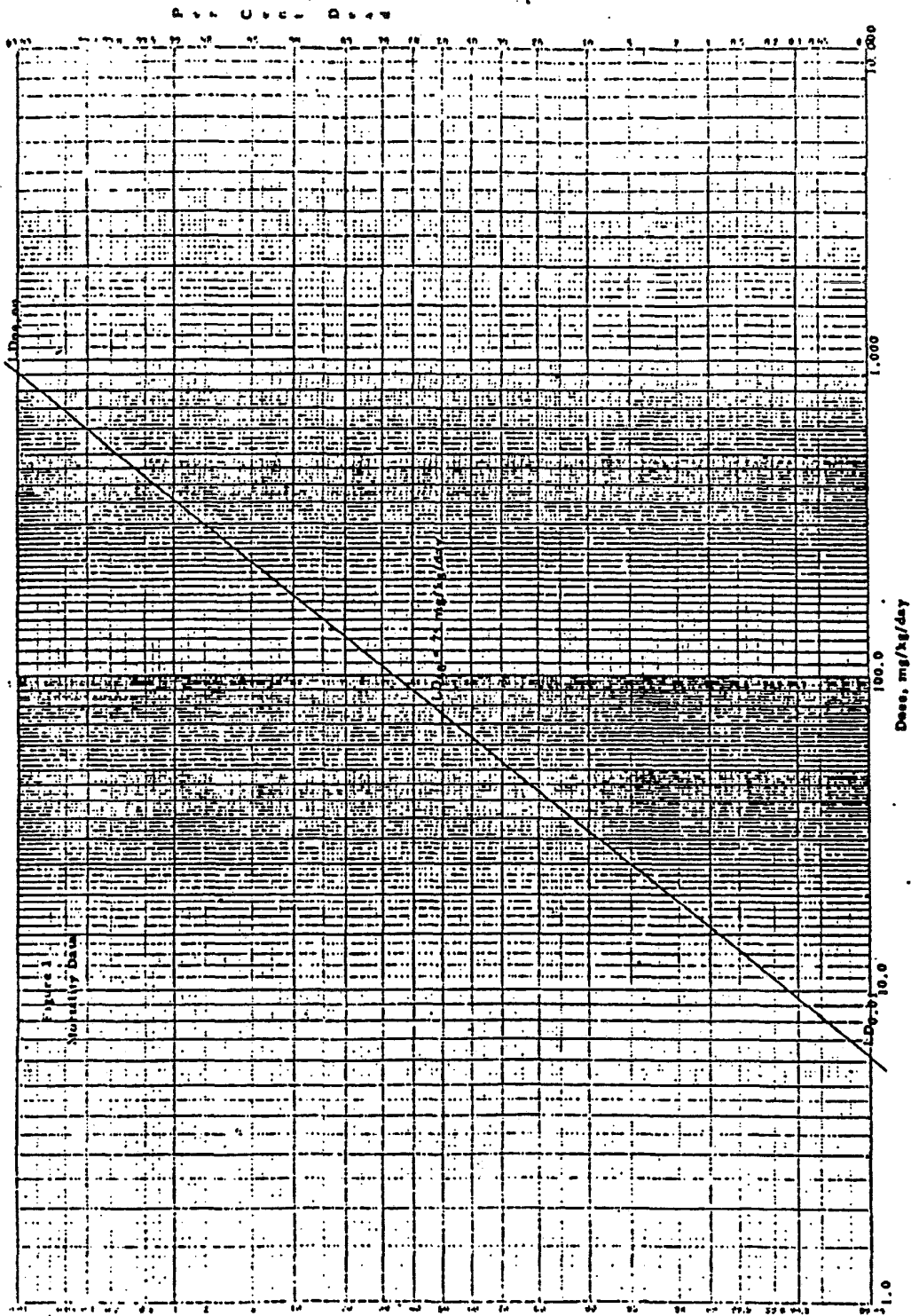
95 Per Cent Confidence Limits of LD₅₀ = 30 to 190 mg/kg/day

Subacute Dermal LD_{0.01} = 6.2 mg/kg/day

Subacute Dermal LD_{99.99} = 915 mg/kg/day

- * The LD₅₀ specifically refers to the dose which, administered each day over a 20-day period, would be expected to kill 50 per cent of the animals so dosed within the 20-day dosing period plus an additional 14 days during which the test material is not applied.

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C. Reactions

1. Behavioral Reactions

No untoward behavioral reactions were noted among the animals receiving doses of 10 mg/kg/day.

Generalized inactivity, weakness, lassitude, and loss of appetite were exhibited by rabbits receiving doses of 50, 100, and 200 mg/kg/day respectively. These symptoms progressed in intensity and eventually ended in profound prostration in moribund rabbits in these dose groups. The surviving female in the 50 mg/kg/day dose group appeared normal by the end of the 14-day observation period. However, the surviving male in the 100 mg/kg/day dose group continued to show slight generalized inactivity and slight loss of appetite at the end of the observation period.

2. Local Skin Reactions

The skin at the application site of animals in the 10, 50, and 100 mg/kg/day dose groups became scaly after the eleventh application of the test material. Slight wrinkling and fissuring were noted after eighteen applications. During the 14-day observation period, desquamation occurred and by the end of this period the skin of the surviving animals appeared normal. Animals receiving doses of 200 mg/kg/day died before any significant untoward local skin reactions could be noted.

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D. Hematologic Studies and Urine Analyses

Values for hematologic data and the results of urine analyses obtained on surviving test animals at the completion of the dosing period did not differ significantly either from those recorded initially or from the corresponding data secured from the treated control animals.

E. Pathology

1. Gross Pathologic Findings

Complete necropsies were performed upon all animals that died during the test and upon all animals sacrificed at the end of the observation period. No significant gross pathologic findings were noted among animals in the 10 mg/kg/day dose group. Animals receiving doses of 50, 100 and 200 mg/kg/day respectively showed liver discoloration (lighter in color) but otherwise no hepatic alterations were seen upon gross examination. All other tissues and organs examined from these animals were comparable to those of treated control animals.

2. Microscopic Pathologic Findings

Microscopic examinations were conducted upon the tissues and organs taken from all sacrificed animals. Animals that died during the test exhibited advanced post-mortem changes. Consequently their tissues and organs were not examined microscopically.

No significant histopathologic alterations were noted in any of the tissues and organs of animals receiving doses of 10 mg/kg/day.

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Moderate hepatic necrosis was noted among surviving rabbits in the 50 and 100 mg/kg/day dose groups. This hepatic change was considered to be a direct effect of percutaneous absorption of the test material. All other tissues and organs examined from these animals were comparable to those of the treated control animals.

NEV 009134

IV. Summary

A. Body Weights

Examination of the body weight data revealed moderate to severe adverse effects among rabbits of Test Groups II, III, and IV (50, 100 and 200 mg/kg/day respectively). The effects were especially pronounced in the moribund animals of these groups. The body weight data of animals receiving 10 mg/kg/day compared well with that of the treated control group.

B. Mortality and Reactions

The 20-day subacute dermal mean lethal dose (LD₅₀) of Aroclor 1254 for the albino rabbit was found to be 75 mg/kg/day.

Generalized inactivity, weakness, lassitude, and loss of appetite were exhibited by rabbits receiving doses of 50, 100 and 200 mg/kg/day respectively. No untoward reactions were noted among animals in the 10 mg/kg/day dose group.

Local skin reactions included scaling, wrinkling, and fissuring among animals receiving doses of 10, 50, and 100 mg/kg/day respectively. These reactions occurred during the second half of the 20-day test period. During the 14-day observation period desquamation occurred and by the end of this period the skin of the surviving animals appeared normal. Animals receiving doses of 200 mg/kg/day died before any significant local skin reactions could be noted.

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C. Hematologic Studies and Urine Analyses

Hematologic data and results of the urine analyses for test group animals compared well with those of the treated control group animals.

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D. Pathology

1. Gross Pathologic Findings

No significant gross pathologic findings were noted among the 10 mg/kg/day dose group. Animals receiving doses of 50, 100, and 200 mg/kg/day respectively revealed liver discoloration (lighter in color). All other tissues and organs examined from these animals were comparable to those of treated control animals.

2. Microscopic Pathologic Findings

No significant histopathologic tissue alterations were noted among animals receiving doses of 10 mg/kg/day. The surviving animals receiving doses of 50 and 100 mg/kg/day respectively revealed moderate hepatic necrosis. This finding was considered to be a direct effect of percutaneous absorption of the test material. All other tissues and organs examined from these animals were comparable to those seen in treated control animals.

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

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Associate Director

March 29, 1963

NEV 009137

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October 11, 1937.

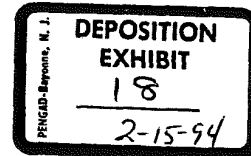
Experimental work in animals shows that prolonged exposure to Arcolor vapors evolved at high temperatures or by repeated oral ingestion will lead to systemic toxic effects.

Repeated bodily contact with the liquid Arcolors may lead to an acne-form skin eruption.

Suitable draft ventilation to control the vapors evolved at elevated temperatures, as well as protection by suitable garments from extensive bodily contact with the liquid Arcolors, should prevent any untoward effect.

In talking with Dr. Kelly before these three paragraphs were written, we agreed that they might as well be phrased so that they could be used not only in the Arcolor booklet, but quoted in correspondence as that may be necessary.

L.A. Watt



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