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PREFACE

Of all the toxicologic research carried out in academic, industrial, and commercial laboratories only a small percentage of the publishable data appears in scientific journals. This has been brought about in part by the fact that manufacturers have been more concerned with meeting requirements for licensure of their products than in the wide dissemination of information, and journals have been reluctant to accept manuscripts dealing solely with toxicology. Those who find it necessary to search the literature for toxicity data are frank to admit that a vast number of scientific periodicals must be searched in order to find the widely scattered data.

In establishing the journal, *Toxicology and Applied Pharmacology,* the Editors hope that it will stimulate investigators to publish extensive toxicologic studies, that it will provide an outlet for papers by those being trained in toxicology, and that it will bring about centralization of important toxicologic research and thus facilitate the work of the investigators.

Biological research and development by both public and private organizations during the past ten years has reached the highest level in our history. The introduction of new chemicals as well as new uses for old ones has created enormous problems. Tolerance for agricultural chemicals must now be established and the criterion is safety for human use. Stockpiling of food for the rapidly increasing population, or of plasma in the event of an atomic disaster has made it imperative to find improved methods of preservation or storage. Man is now living in confined spaces for long periods of time, as was recently demonstrated on expeditions of the submarines *Nautilus* and *Sea Wolf* and this requires a re-evaluation of the problems of air pollution. To travel in outer space, the area in which man will be confined will be even smaller. These and many other problems must be resolved and toxicology will contribute to the solution.

Toxicology is on the threshold of emerging into a scientific discipline of its own, for the number of problems which will require extensive toxicologic research are far greater than can be resolved by those working in related fields. The responsibility of the toxicologist is greater than ever before and if he is to meet this challenge he will of necessity have

to be specially trained as a toxicologist rather than acquire his knowledge of the field as a part of other scientific disciplines.

Although the increasing number of scientific journals being offered today may give cause for concern, the greatly expanded research program in toxicology has made evident the need for a journal that will make its pages available to scientists in many related fields and will at the same time bring together in one medium papers dealing with toxicology and those which apply the principles of pharmacology.

The proposed journal has received cordial support both in this country and abroad and we trust that it will have a long and useful life.

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The Subacute Toxicity of Four Organic Phosphates to Dogs

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The toxicity of the four organic phosphates chosen for this study has been given only limited attention in the literature and most of this has been of an acute nature. Two of these compounds, methyl parathion (*O,O*-dimethyl *O*-*p*-nitrophenyl phosphorothioate), and Diazinon [*O,O*-diethyl-*O*-(2-isopropyl-4-methyl-6 pyrimidinyl) phosphorothioate] may legally appear as residues in some agricultural commodities, while the other two materials Dipterex (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate) and Chlorthion [*O*-(3-chloro-4-nitrophenyl) *O,O*-dimethyl phosphorothioate] may be used in spray formulations and other applications only where no food residues result. All four of these compounds are *in vivo* inhibitors of cholinesterase and all present an acute toxicity syndrome typical of this entire class of materials, i.e., cholinergic in character. In view of the widespread use of these compounds and the relative paucity of toxicological data of a subacute nature, it was considered pertinent to study them in the dog at low levels over a three-month period, while following the red blood cell and plasma cholinesterase levels.

Du Bois and Cotter (1955) in a study of Dipterex found this material to have a relatively low acute toxicity, the oral LD_{50} in rats being 450 mg/kg, while Hagan (1958) found it to be 316 mg/kg and Deichmann and Lampe (1955), 500 mg/kg. Subacute observations by Du Bois and Cotter (1955) indicated that intraperitoneal daily doses of 50 mg/kg to rats for 60 days resulted in survival of all animals, while higher dosages produced increased mortality. Cholinesterase determinations by the same investigators on brain, serum, and submaxillary glands of rats following

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single acute intraperitoneal administration of 25, 50, and 125 mg/kg indicated transient depression at all levels and in all three tissues with relatively complete return to pretreatment levels within 5 hours. Subacute toxicity of Dipterex in two dogs is reported by Deichmann and Lampe (1955), who found, when feeding 42 mg/kg (i.e., 10% of a lethal oral dose) 6 days a week for three months, that the average plasma cholinesterase had fallen to 59.5% of the control level, while no overt signs of systemic intoxication were noted.

The second compound to be considered is Chlorthion. This material also has been studied by Du Bois and co-workers (1953), who found the oral LD_{50} in rats was 1500 mg/kg and the intraperitoneal LD_{50} was 750 mg/kg in the same species. Hagan (1958) found the oral LD_{50} to be 989 mg/kg. Subacute studies by Du Bois *et al.* (1953) indicated that 50 mg/kg per day when administered daily by the intraperitoneal route could be tolerated for 60 days, while increased levels decreased survival time. Large single intraperitoneal doses of Chlorthion, i.e., 250 mg/kg, produced in rats up to 80% inhibition in the cholinesterase levels of three tissues tested (i.e., brain, serum, and submaxillary gland) within 12 hours.

The third of the series to be considered is methyl parathion. The acute intraperitoneal LD_{50} for this material in rats is 3.5 mg/kg, according to Du Bois and Coon (1952). The oral LD_{50} noted by Hagan (1958) was 17.2 mg/kg. Thus it appears that the acute toxicity of methyl parathion is roughly the same as that of parathion, though according to Hazleton (1955) parathion is a more potent cholinesterase inhibitor.

The fourth and last of the compounds under study is Diazinon. This material, while of moderate acute oral toxicity, is an extremely potent cholinesterase inhibitor. The oral LD_{50} in the rat is 264 mg/kg (Bruce *et al.*, 1954).

Feeding levels in this study in each case were selected so as to determine the highest dosage which, when fed over the 90-day period, would produce no cholinesterase depression. Additional levels which would produce statistically significant depression in cholinesterase activity were also utilized.

MATERIALS AND METHOD

Two dogs, one male and one female, of mixed breeds weighing 6–10 kg and housed in individual metabolism cages, were placed on each of three levels of methyl parathion, Diazinon, and Dipterex, while a fourth level

was added in the case of Chlorthion. Four control dogs were studied simultaneously, except for Diazinon where five were used. The food consisted of a commercial ground dog chow to which was added the proper concentration of a stock mixture containing the phosphate in question, and water was allowed ad libitum.

The organic phosphates in all cases were technical grade except for Diazinon which was the powdered formulation 25W. This material was 24.1% pure. With the exception of Diazinon, which was corrected, the other compounds were considered to be 100% pure. All animals were acclimated to the laboratory environment for at least a week. During the following 4-week period five control cholinesterase determinations were made by the method of Michel (1949) as modified by Frawley and Fuyat (1957) on samples of blood drawn by external jugular puncture.

Following the control period, feeding of the following levels of organic phosphates was instituted: Dipterex at 50, 200, and 500 ppm; Chlorthion at 0.5, 2, 5, and 15 ppm; methyl parathion at 5, 20, and 50 ppm; and Diazinon at 0.25, 0.75, and 75 ppm.

Control animals were given the stock diet, and cholinesterase determinations were made upon both the plasma and red cells for all animals at the end of the first week and every second week for a 12-week feeding period. An 8-week posttreatment control period followed in all cases except with Diazinon, where a 6-week period was observed. During this time the animals were placed on a control diet and the blood cholinesterase followed.

Statistical evaluation of weekly data consisted of determining whether the experimental delta pH values varied in excess of twice the standard deviation of the pretreatment control values. When such was the case these values were considered to be statistically significant.

RESULTS AND DISCUSSION

The results of this investigation are summarized in Figs. 1–4.

The feeding of Dipterex (Fig. 1) in the total diet at three levels, 50, 200 and 500 ppm, produced significant depression in both plasma and red-cell cholinesterase within 2 weeks at 500 ppm, and borderline depression at 200 ppm, while no depression was noted at 50 ppm. The rate of fall in cholinesterase noted in plasma was more rapid than in the case of the red cell; the peak depression reached in the plasma at the high level after 6 weeks on the toxicant was to 40% of pretreatment

control, while it was 55% at the eighth week in the red cell. There was a tendency for the plasma cholinesterase to start a gradual return toward normal after 8 weeks, even though feeding of the toxicant continued. The terminal observation on plasma cholinesterase at 500 ppm, where we found a depression of 55% of the pretreatment control value, agreed remarkably well with the final average level of 59.5% observed by Deichmann and Lampe (1955), who fed 42 mg/kg per day for a 6-day week to two dogs for three months. Assuming a semisolid diet, this dosage

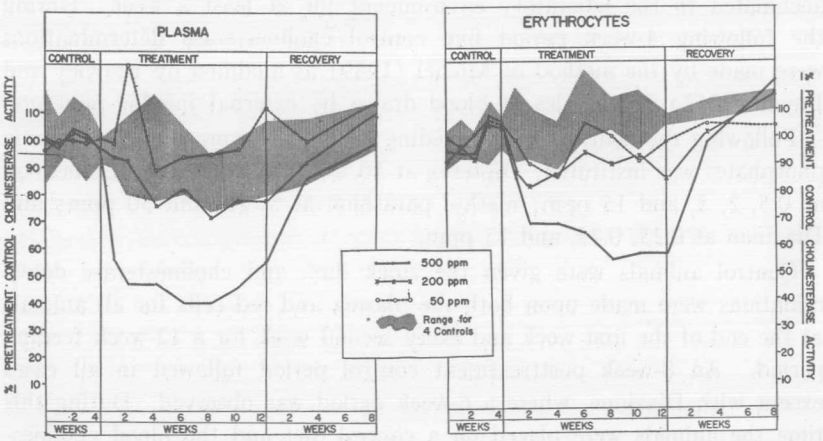

FIG. 1. Effect of feeding diets containing Dipterex on plasma and erythrocyte cholinesterase activity of dogs.

was approximately 468 ppm. The "no effect" level for Dipterex in the dog would appear to be between our two experimental dosages of 50 and 200 ppm.

Analysis of Fig. 2 for Chlorthion indicates that dosages up to 15 ppm in the total diet produce only questionable cholinesterase depression; one point at the eighth week is significant ($p < 0.05$), and this only in the erythrocytes. None of the lower levels, 0.5, 2, or 5 ppm, produced significant depression of cholinesterase. From this it appears that 15 ppm is borderline in dogs and that 5 ppm is a "no effect" level.

Figure 3 indicates cholinesterase activity for methyl parathion at all levels. Both of the higher dosage levels of 50 and 20 ppm produced significant depression in both plasma and red-cell cholinesterase. Based upon the data at the termination of the experiment, it appears that the

toxicant at both higher levels has not reached the peak of inhibition. It is impossible to say how much farther it would fall if given additional time. In this respect it would seem that the effects of this toxicant tend to be more cumulative than in the other cases. The highest level which

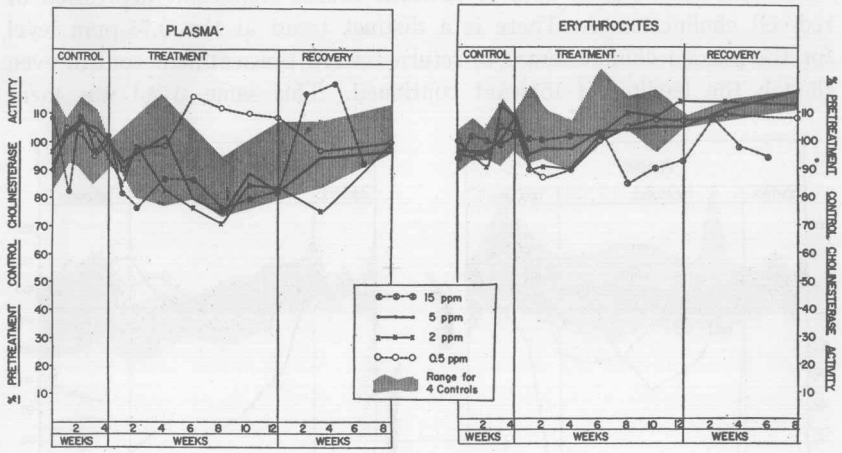

FIG. 2. Effect of feeding diets containing Chlorthion on plasma and erythrocyte cholinesterase activity of dogs.

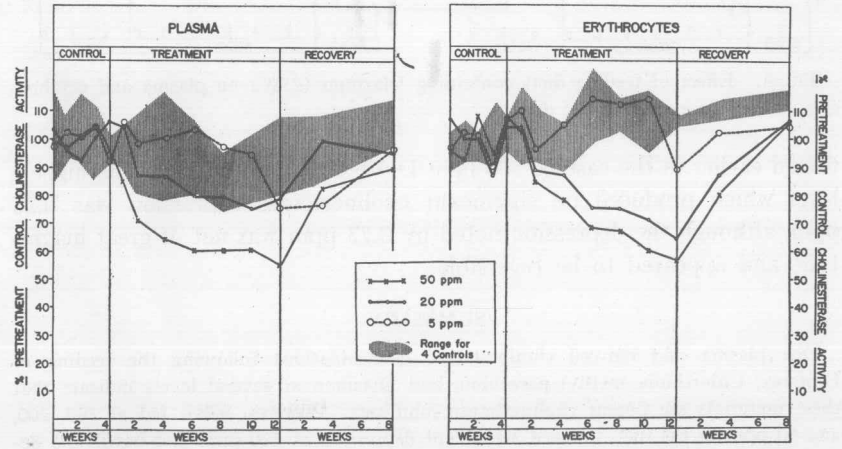

FIG. 3. Effect of feeding diets containing methyl parathion on plasma and erythrocyte cholinesterase activity of dogs.