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Toxicity of Carbamates for Mammals

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Toxicity studies have been carried out with a number of monomethylcarbamates, most of which reached an advanced stage in the World Health Organization insecticide evaluation programme. Both quantitative and qualitative distinctions have been found between the carbamates studied, and certain common characteristics that distinguish them in several important aspects from organophosphorus insecticides have been demonstrated.

During the last 10 years the toxic properties of carbamates have been studied at the Institute for Medical Research, Zagreb, Yugoslavia.[3] *In vitro* and *in vivo* studies have been carried out and, in some instances, comparisons with organophosphorus compounds have been performed. This paper deals with monomethylcarbamates that entered the WHO insecticide evaluation programme (Table 1) and outlines the results of determinations of acute toxicity, of persistence in the blood, and of the effects of different rates of infusion into the jugular or portal veins of rats. A particular objective of the latter studies was to determine the doses necessary to produce a given symptom (and/or cholinesterase depression) or death. A brief account is also given of studies carried out in volunteers to develop a suitable method for measuring exposure to propoxur.

STUDIES IN EXPERIMENTAL ANIMALS

Relationship between acute toxicity and anticholinesterase activity

Male rats were usually used for the determination of LD_{50} values, except for propoxur and *m*-isopropylphenyl methylcarbamate, whose toxicity was determined in female rats. No significant differences in LD_{50} values between the two sexes were found. Three routes of administration were employed; the results are summarized in Table 1.

In addition to typical symptoms of anticholinesterase poisoning, five carbamates (carbaryl, *m*-isopropylphenyl methylcarbamate, phenyl methylcarbamate, 3,5-diisopropylphenyl methylcarbamate, and Landrin †) produced a transient but very pronounced anaesthetic effect immediately following the intravenous administration of a dose near the LD_{50} (Wilhelm & Vandekar, 1966). Lethal doses caused deep anaesthesia with severe dyspnoea, and respiration ceased within a few minutes. If artificial respiration was applied for 2–5 minutes within the first minute of apnoea spontaneous respiration resumed and the animals slowly recovered from anaesthesia and cholinergic symptoms gradually developed. Thus, the pronounced anaesthetic effect with respiratory failure was the most critical sign determining the intravenous toxicity of these five compounds. A similar but much less pronounced anaesthetic effect was also produced by the intraperitoneal injection of four carbamates (carbaryl, *m*-isopropylphenyl methylcarbamate, phenyl methylcarbamate, and 3,5-diisopropylphenyl methylcarbamate). However, no such effect was observed when these compounds were administered orally.

It should be noted that the narcotic effects described above were produced only by carbamates of relatively low toxicity and closely resembled those caused by certain organophosphorus compounds of low toxicity (Vandekar, 1957). The mechanism of action is not certain, but Heath (1961) put forward the reasonable hypothesis that, since these effects are marked only after injection into the blood stream, the concentrations produced are sufficiently high to block nerve conduction and motor endplates, the narcotic block of conduction being attributed to a block in sodium-ion transport across the axon membrane.

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† Names against which this symbol oppears are identified in the Glossary on pages 445-446.

Table 1

Acute toxicity of monomethylcarbamates and their inhibition of AChE

Methylcarbamate	Common or proprietary name	Male rats				Bovine erythrocyte AChE I_{50} (M)
		Intravenous LD_{50} (mg/kg)	Intravenous ED_{50} (mg/kg)	Intraperitoneal LD_{50} (mg/kg)	Oral LD_{50} (mg/kg)	
1-naphthyl	carbaryl	41.9 [a]	1.42	200 [b]	250	1.5×10^{-6}
o-isopropylphenyl	—	66.0 [a]	—	142 [b]	374 [c]	1.2×10^{-6}
o-isopropoxyphenyl	propoxur	10.6	0.334	30.0	100	7.0×10^{-7}
m-isopropylphenyl	—	3.15	0.168	14.2	39.2 [c]	5.6×10^{-8}
2-chloro-4,5-dimethylphenyl	carbanolate	3.00	0.144	11.2	60.0 [c]	7.3×10^{-8}
phenyl	—	13.6 [a]	—	357 [b]	540 [c]	8.8×10^{-5}
3,5-diisopropylphenyl	—	29.7 [a]	—	267 [b]	1 000 [c]	1.9×10^{-7}
3,4,5-trimethylphenyl	Landrin	31.8 [a]	1.5	94.4	236 [c]	9.0×10^{-7}
4-benzothienyl	Mobam	24.8	0.707	40.8	336	—
m-cym-5-yl	promecarb	5.30	0.189	27.2	125	8.8×10^{-8}

[a] Pronounced anaesthetic effect observed.
[b] Slight anaesthetic effect observed.
[c] Data from the Medical Research Council Laboratories, Carshalton, England.

Simeon (1966) compared the acute toxicity for rats of 9 monomethyl carbamates, administered in a single intravenous injection, with their anticholinesterase activity determined on purified bovine erythrocyte cholinesterase (see Table 1), the enzyme being incubated with a given concentration of inhibitor for 30 minutes at 25°C and its activity subsequently measured titrigraphically at the same temperature and at pH 7.4. Good correlation was found between the I_{50} and the intravenous LD_{50} values, the correlation coefficient being 0.89. Such a degree of correlation should be regarded as particularly high in view of the small number of compounds compared and the relatively narrow range of their I_{50} values (8.8×10^{-5} to 5.6×10^{-8} M) and LD_{50} values (3.0–136.0 mg/kg) and in view of the fact that in some instances the correlation was likely to be vitiated to a certain extent by the presence of the narcotic effect described above. These results indicate that, unlike their (nonsynergized) insecticidal activity (Fukuto et al., 1962), the acute toxicity of carbamates for rats follows their anticholinesterase activity. In mammals, the rate of metabolism of this type of carbamate is, apparently, not a factor in determining their LD_{50} values, and their toxicity can therefore be predicted from the degree to which they inhibit cholinesterase *in vitro*.

Symptoms and persistence of inhibitor in the body

A straight comparison of the type and duration of symptoms was carried out with 15 monomethylcarbamates by injecting groups of 4 rats intravenously with a single i.v.-LD_{50} dose (Wilhelm & Vandekar, 1966; Wilhelm, unpublished data). The intensity of different cholinergic symptoms varied considerably from one compound to the other, indicating marked differences in their distribution in the body following intravenous injection. At the same time, differences in the duration of cholinergic symptoms were recorded: with some compounds symptoms ceased within 45 minutes, while with others they lasted for more than 2–3 hours. These differences led the authors to study the persistence of the inhibitor in the body. This was carried out by determining the anticholinesterase activity of rat serum at given intervals after the intravenous injection of a single sublethal dose of carbamate. A procedure analogous to that used in the study of some dimethyl phosphate esters was employed (Vandekar & Heath, 1957), the anticholinesterase activity of rat serum being estimated on human erythrocyte ChE *in vitro*. It has been found that the carbamates inducing symptoms of short duration (e.g., propoxur, *m*-isopropylphenyl methylcarbamate, carbanolate, and promecarb) persisted in the blood for only 1–2

hours, while those inducing prolonged symptoms (e.g., carbaryl, *o*-isopropylphenyl methylcarbamate, phenyl methylcarbamate, and Landrin †) persisted in the blood for more than 6 hours.

Similar results were obtained when carbamates were administered orally in equitoxic doses (Wilhelm, unpublished data): compounds inducing symptoms of prolonged duration (4–5 hours), such as carbaryl, persisted in the blood for a considerably longer period than did those inducing symptoms of shorter duration (about 2 hours), such as propoxur. As described below, it was possible, by using a similar method in studies on human volunteers, to determine the presence of free inhibitor in the blood for several hours after the ingestion of relatively small doses of carbamate.

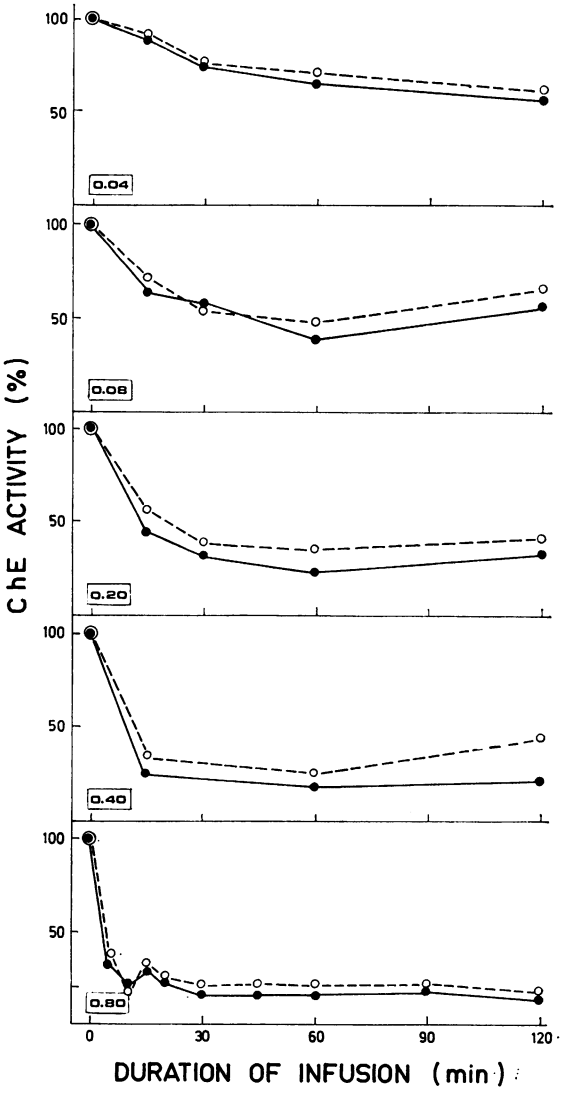
Relationship between symptoms and cholinesterase activity

In a series of experiments, brain and plasma ChE activity was determined in rats to which propoxur was administered intramuscularly at different dosages, the animals being sacrificed at the moment the first symptoms appeared or at a given time after injection (Pleština & Vandekar, 1966). At dosages that did not produce any noticeable symptoms (0.25–1.0 mg/kg), the activity of both brain and plasma ChE was reduced by varying amounts, down to about 60% of the normal level. The dose at which a very slight tremor occurred (2.0 mg/kg) reduced the brain and plasma ChE activities to 47% and 49% of the normal levels, respectively, the animals being sacrificed immediately after this sign had been observed. At higher dosages (10.0 and 50.0 mg/kg) the degree of inhibition of both brain and plasma cholinesterase closely followed the severity of the symptoms that were produced, the brain ChE usually showing 5–15% greater inhibition than the plasma ChE.

In another series of experiments the relationship between the brain and plasma cholinesterase activities and the degree of symptoms was studied during the infusion of propoxur into the jugular vein (Pleština, unpublished data). Different rates of infusion, ranging from 0.04 to 0.80 i.v.-LD_{50}/hour, were used. At given time intervals the animals were sacrificed and their brain and plasma ChE levels were determined spectrophotometrically (Ellman et al., 1961), the analytical procedure being completed within 12 minutes after the animals had been decapitated. Three main symptoms (tremor, muscle fasciculations, and salivation) were regularly recorded and were arbitrarily classified according to 5 degrees of intensity. The mean brain and plasma ChE activities in 4–5 animals, expressed as percentages of the mean levels in 40 normal animals, are shown in Fig. 1. These results show good correlation between the activities of the two enzymes and good agreement between the degree of enzyme depression and the

Fig. 1

Effect of propoxur on cholinesterase activity in rats *



● brain cholinesterase

○ plasma cholinesterase

* Propoxur was infused into the jugular vein at rates of 0.04, 0.08, 0.20, 0.40, and 0.80 i.v.–LD_{50}/h. Each point represents the mean of the levels in 4–5 animals.

intensity of symptoms, the onset of symptoms being recorded only after the brain ChE activity dropped to about 50% of normal. It may be noted that during the second hour of infusion a steady state was reached so far as enzyme inhibition and severity of symptoms were concerned.

Ratio between LD_{50} and ED_{50} values

It has been shown from the kinetics of the inhibition of cholinesterase by monomethylcarbamates and organophosphorus compounds that the LD_{50} is likely to be a much greater multiple of the dose causing signs of poisoning for the carbamates than for the organophosphates. The expected difference was demonstrated by a comparison of LD_{50} and ED_{50} values, intravenous and intramuscular, of a number of monomethylcarbamates and two organophosphorus compounds (Vandekar et al., 1965). The slightest evoked tremor was selected as the most reliable sign of poisoning in rats from which to estimate the ED_{50} values. Carbamates gave much greater LD_{50}/ED_{50} ratios (20–35 for intravenous administration, cf. Table 1) than the two organophosphorus compounds studied, paraoxon and its dimethyl homologue, which gave the ratios 4.2 and 2.5, respectively. Similarly, in experiments performed on dogs with *m*-isopropylphenyl methylcarbamate and later with propoxur and promecarb (Svetličić, personal communication) a high LD_{50}/ED_{50} ratio was observed, the initial symptoms including both tremor and increased salivation.

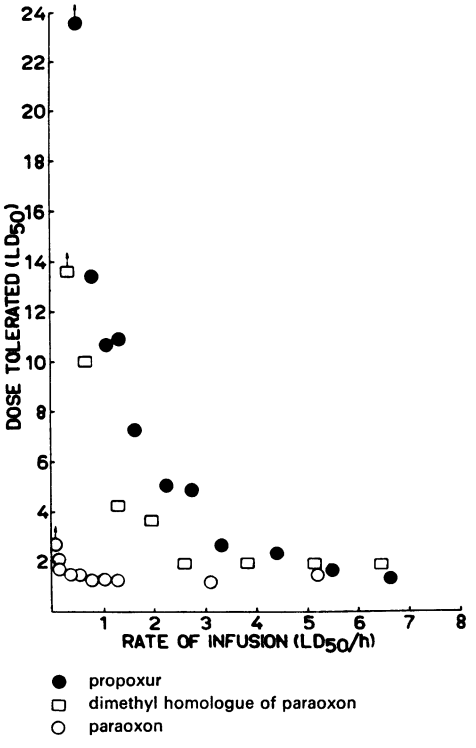
Goldberg et al. (1963) have reported for *m*-isopropylphenyl methylcarbamate a similar (25 : 1) ratio between the intraperitoneal LD_{50} and the dose causing behavioural changes in 50% of a group of specially trained rats.

Tolerated dose at different rates of infusion into general and portal circulation

Proceeding along the lines described above, the tolerated dose of carbamates at different rates of infusion was compared with that of organophosphorus compounds. Thus, it was shown that slowing the rate of intravenous infusion of the three tested carbamates (propoxur, promecarb, and *m*-isopropylphenyl methylcarbamate) led to a large increase in the doses producing death, animals reaching, in the course of infusion, a " stage of equilibrium " of symptom intensity, which lasted for 10 or more hours (Vandekar & Fajdetić, 1966). This is illustrated in Fig. 2, in which results obtained with propoxur are compared with those obtained with two organophosphorus compounds, paraoxon and its dimethyl homologue. While a large increase in the tolerance of propoxur was produced when the rate of infusion was reduced to less than 3 LD_{50}/hour, the rate of infusion of the dimethyl phosphate had to be reduced to less than 2.0 LD_{50}/hour and that of the diethyl phosphate to less than 0.1 LD_{50}/hour to produce an increase in the dose tolerated.

Fig. 2

Doses of propoxur, paraoxon, and the dimethyl homologue of paraoxon causing death of rats *



* The compounds were infused into the jugular vein at different rates. Each point represents the mean of the levels observed in 6 animals. The levels are expressed in terms of i.v.–LD_{50} values, and all were determined in the same laboratory.

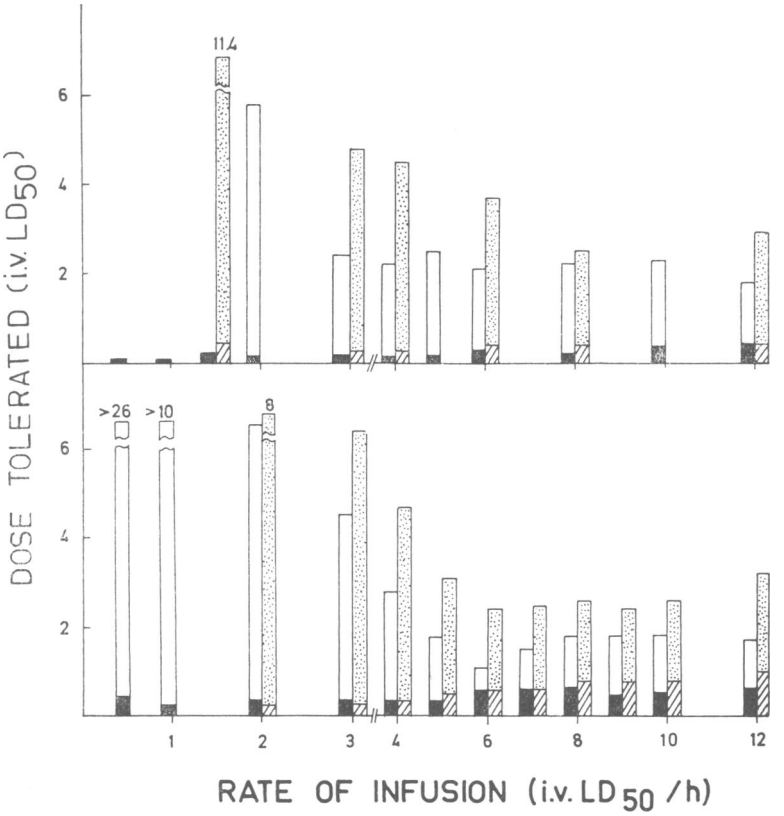
It was possible to make a further distinction between carbamates and organophosphorus compounds in this type of experiment. Whereas a decrease in the rate of infusion of carbamates was accompanied by a remarkable increase in the lethal dose, no increase in the dose necessary to produce the first symptoms was recorded at the rates of infusion employed. Vandekar & Fajdetić (1966) showed, for a number of carbamates injected into the jugular vein of the rat, that the ratio between the doses producing death and those producing the first notice-

able symptoms (tremor or fasciculations) became larger as the rate of infusion was reduced, reaching values around 50. Similar experiments with paraoxon, in which the rates of infusion were 0.059–0.29 mg/kg/h, revealed constant ratios between the doses causing death and those producing the first symptoms, the first symptoms being observed only after about half of the lethal dose had been injected.

Tolerance of the monomethylcarbamates was also studied at different rates of infusion into the portal circulation, and this was compared with the tolerance observed when they were infused into the jugular vein (Pleština, unpublished data). The results obtained with propoxur and promecarb are shown in Fig. 3. It may be noted that the amount tolerated until the moment of appearance of the first cholinergic symptoms was approximately the same for both compounds, regardless of the rate and route of infusion. The amount of carbamate required to kill the rats was dependent on both the rate and the route of infusion, larger amounts being tolerated by infusion via the portal vein, particularly at the lower rates of infusion. It should be pointed out, however, that much more pronounced differences between the two routes of infusion were observed in similar studies with organophosphorus compounds belonging to the group of direct ChE inhibitors: the amounts tolerated via the portal vein were about five times those tolerated via the jugular vein. These results indicate that, with the carbamates studied, the role of the liver, while demonstrable, is relatively small and that the main factor contributing to the large increase in tolerance at lower rates of infusion is probably the rapid

Fig. 3

Lethal and symptom-producing doses of propoxur (top) and promecarb (bottom) for the rat *



* The black columns indicate doses producing first symptoms in, and the white columns doses causing death of, rats subjected to ntrajugular infusion. Diagonally shaded columns indicate doses producing first symptoms in, and stippled columns doses causing death of, rats subjected to intraportal infusion. Each dose represents the mean for 3–6 rats.

reactivation of the inhibited cholinesterase, which "competes" with the inhibition process during infusion. The fact that the dermal toxicity of a number of monomethylcarbamates has been shown to be markedly lower than their toxicity when administered by other routes (Gaines, 1960, 1969) can be attributed mainly to the same phenomenon.

STUDIES IN VOLUNTEERS

Useful as they are, experiments on laboratory animals are of limited use in evaluating methods for measuring exposure to a compound (or the extent to which it is absorbed), nor are they of value in certain other areas, such as clarifying the significance of blood cholinesterase levels in relation to different conditions of exposure to a given compound. Field studies on persons exposed to insecticidal carbamates and studies in volunteers may provide the answer to such practical problems. Some studies in volunteers that have been carried out with the particular objective of evaluating methods for measuring blood cholinesterase levels in persons exposed to propoxur (Vandekar, unpublished report to WHO, 1966; Pleština, 1968; Pleština, unpublished data) will be briefly described.

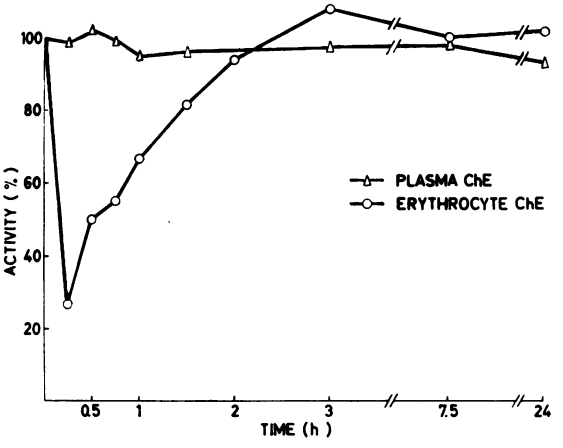
Propoxur (95% pure, recrystallized before use) was given orally to healthy volunteers—investigators who were actively involved in the study—either in a single dose or in several doses over a certain period of time, usually 2 hours—the latter imitating to a certain extent the occupational exposure to insecticides. Different methods for cholinesterase assay were compared; signs and symptoms, if any, were correlated to cholinesterase activity; the persistence of the inhibitor in the blood was studied; and the excretion of phenol derivatives in the urine was determined.

Single dose

A 42-year-old male volunteer (90 kg body weight) ingested 1.5 mg of propoxur per kg of body weight about 2 hours after his usual "Continental" breakfast. This dose was similar to that given to two male subjects by Dawson et al. (1964) in a study undertaken to develop a quantitative method for determining the metabolites of this insecticide excreted in urine. As shown in Fig. 4, a rapid fall in erythrocyte cholinesterase activity but no depression in plasma cholinesterase was observed, both enzymes being determined spectrophotometrically (Ellman et al., 1961). This is consistent with the difference observed in the affinity of propoxur for

Fig. 4

Effect of propoxur on cholinesterase levels in man *



* The cholinesterase levels were determined in a single subject following the administration of 1.5 mg of propoxur per kg of body weight.

the two enzymes *in vitro*, the I_{50} values for erythrocyte and plasma cholinesterase being 4.6×10^{-7} M and 2.3×10^{-5} M, respectively (Wilhelm, 1967). The lowest erythrocyte cholinesterase level (27.0% of normal) was observed 15 minutes after ingestion. No signs were observed at that time, but moderate discomfort, described as "pressure in the head", was present. Blurred vision and nausea developed 3 minutes later, and 20 minutes after ingestion the subject was pale and his face was sweating; his pulse rate was 140/min (before ingestion it was 76) and his blood pressure was 175/95 mm Hg (before ingestion it was 135/90). Within the next 10 minutes pronounced nausea, with repeated vomiting and profuse sweating, developed. These symptoms lasted, with no change in intensity, from about the 30th until about the 45th minute; during this period erythrocyte cholinesterase activity recovered from a level of 50.4% to one of 55.5% of its normal value. One hour after ingestion the subject was feeling better and his sweating was less pronounced, but he still felt nauseated and tired. His pulse and blood pressure were found to be normal 10 minutes later, and 2 hours after ingestion he was feeling well and he had a complete lunch and dinner without discomfort. The rapid disappearance of symptoms was consistent with the further rapid recovery of erythrocyte cholinesterase activity.

The persistence of inhibitor in the blood was determined by means of the procedure described

above. The inhibition of erythrocyte cholinesterase *in vitro* by plasma samples collected 0.5, 2.0, and 7.5 hours after ingestion indicated the presence of propoxur in the plasma at molar concentrations of 5.0×10^{-7}, 3.2×10^{-7}, and 1.5×10^{-7}, respectively.

The method described by Dawson et al. (1964) was used to determine phenol derivatives in the urine, and the results are shown in Table 2. The amount of *o*-isopropoxyphenol excreted in the urine over a 24-hour period corresponded to about 45% of the total dose of propoxur ingested. As abundant vomiting started 23 minutes after ingestion it may be assumed that a relatively large proportion of the ingested dose did not reach the circulation. Excretion was very rapid, 81% of the total amount excreted being found in the first 2 samples, which were collected within 4¾ hours after ingestion.

Table 2

Excretion of phenol derivatives by a human subject following a dose of 1.5 mg of propoxur per kg of body weight

Time after ingestion	Volume of urine (ml)	Concentration in urine (μg/ml)
Pre-exposure value	55	20.0
1 h 50 min	108	177.5
4 h 45 min	107	195.6
7 h 10 min	250	37.8
8 h 35 min	196	21.3
8 h 35 min to 24 h	790	24.3

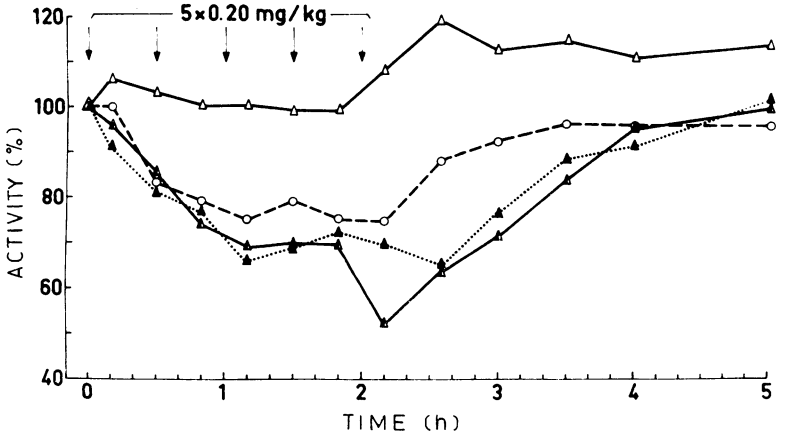
In another experiment a single dose of 0.36 mg/kg produced a rapid fall in erythrocyte cholinesterase to 57% of the normal level within 10 minutes, short-lasting (about 5 minutes) stomach discomfort, blurred vision, and moderate facial redness and sweating. The cholinesterase recovered to its normal value within 3 hours.

Repeated doses

In a number of experiments carried out to study the effect of storage of inhibited blood samples under various conditions, volunteers took 5 doses of either 0.15 mg/kg or 0.20 mg/kg at half-hourly intervals. In each subject a symptomless depression of erythrocyte cholinesterase to about 60% of the normal level was observed, recovery being rapid after the cessation of dosing (Fig. 5). Similarly, pronounced—and, as a rule, symptomless—daily depression and reactivation of cholinesterase was observed in persons who were occupationally exposed to this insecticide (Vandekar et al., 1968).

Fig. 5

Comparison of cholinesterase levels as determined spectrophotometrically and tintometrically *



* The levels were measured in a single human subject who was given 5 doses of 0.20 mg of propoxur per kg of body weight at 30-min intervals.

It may also be noted in Fig. 5 that whole-blood cholinesterase levels determined by the tintometric method, a field method designed originally for determining exposure to organophosphorus compounds (Edson, 1958), were in reasonably good agreement with the erythrocyte cholinesterase levels determined by the Ellman spectrophotometric method. A slight adjustment was, however, made to render the tintometric method suitable for determining the degree of cholinesterase inhibition in persons exposed to carbamates: to diminish the reactivation of carbamoylated enzyme, the substrate was added to the reaction mixture immediately after the addition of the blood sample. The usefulness of methods for determining the degree of exposure to propoxur has been discussed by Wright et al. (1969) on the basis of experience gained in a number of village-scale and large-scale field trials carried out with this compound.

The studies in volunteers have shown clearly that, while a single relatively small oral dose (0.36 mg/kg) of propoxur may produce symptoms of short duration, higher doses may be tolerated without symptoms (although there is appreciable inhibition of erythrocyte cholinesterase) if they are divided into portions that are taken within a relatively brief period.

CONCLUSIONS

Certain common characteristics of monomethylcarbamates distinguish them in several important respects from organophosphorus compounds. The differences between the two groups of compounds are attributable to the fact that the inhibition of cholinesterase produced by carbamates is of short duration in comparison with that produced by organophosphorus compounds. Although carbamates of low toxicity induce symptoms of somewhat longer duration than do more toxic ones when applied at equilethal dosages, there is a rapid recovery in cholinesterase activity and a rapid disappearance of symptoms after the cessation of exposure, in marked contrast to poisoning by organophosphorus compounds.

Being direct inhibitors of cholinesterase, carbamates produce rapid inhibition in the early stages of exposure; it is, however, difficult to produce a severe degree of inhibition, because the rate of reactivation of the inhibited cholinesterase approaches that of inactivation. Thus, as demonstrated by infusion experiments and LD_{50}/ED_{50} ratios, the lethal dose of a carbamate is a considerably greater multiple of the dose causing the first signs of poisoning than is the case for organophosphorus compounds. Consequently, in cases of occupational overexposure to carbamates, an early warning of poisoning—the appearance of slight but unmistakable symptoms—may be expected long before a dangerous dose is absorbed. At a certain rate of absorption—for propoxur, about 0.30 mg/kg/h orally—exposure may be continued for long periods without danger of poisoning.

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DISCUSSION

BARNES: Did any of the volunteers who were given carbamates complain of muscle weakness?

VANDEKAR: Yes. Both volunteers who complained of symptoms mentioned tiredness and weakness; one of them specifically described it as " muscle weakness ".

HOLAN: The symptoms that occurred in the human volunteers sound psychologically similar to those that are produced by acute anxiety. Was a double blind study carried out or did the subjects know that they were taking the pesticide?

VANDEKAR: Profuse sweating, nausea, and vomiting are also observed in some spray-men who are over-exposed in the field. They cannot be attributed to acute anxiety. The volunteer was given no information on the biochemical findings until after his recovery.