Toxicology, 22 (1982) 311—317
Elsevier/North-Holland Scientific Publishers Ltd.

STOMACH ABSORPTION OF INTUBATED INSECTICIDES IN FASTED MICE

SAAD AHDAYA and F.E. GUTHRIE

Toxicology Program/Entomology Department, North Carolina State University, Raleigh, NC 27650 (U.S.A.)

(Received August 10th, 1981)
(Revision received November 24th, 1981)
(Accepted December 1st, 1981)

SUMMARY

Eight ^{14}C-labeled insecticides representing diverse chemical classes were intubated into fasted mice whose stomachs were ligated at the pylorus. Absorption through the stomach was measured at 3 time intervals over a 60-min period and compared to similar absorption studies in the entire gastrointestinal tract. The percent of stomach absorption (as contrasted to total gastrointestinal absorption) varied from 29% (carbaryl) to 10% (nicotine). Distribution following stomach absorption was found to be similar to that in the gastrointestinal tract.

INTRODUCTION

Although the stomach is generally considered a minor absorptive organ, its role in absorption of hazardous chemicals has been little investigated. Karel [1] has reported that gastric absorption may be grossly underestimated with regard to physiologically active compounds present in small amounts. Several investigators [2—5] have reported studies on gastric absorption, most dealing with weak bases or acids and limited primarily to drugs. It was concluded that absorption of drugs appears to involve passive diffusion, and the rates were governed by such physicochemical properties as lipid solubility, molecular weight, and degree of ionization. Gastrointestinal absorption of a number of insecticides has been reported recently [6] and a review of absorption of carbamates, including gastric absorption, has also been published [7]. To increase understanding of the process of gastric

Address all correspondence to: F.E. Guthrie, Department of Entomology, North Carolina State University, Raleigh, NC 27650, U.S.A.
Abbreviation: LSR, least significant ratio.

0300-483X/82/0000—0000/$02.75

absorption as related to insecticides, a comparison was made of absorption through the ligatured stomach and absorption through the gastrointestinal tract using 8 insecticides with widely differing physicochemical properties.

MATERIALS AND METHODS

Chemicals

Radioactive compounds used were as follows: [^{14}C]malathion (succinyl-labeled, spec. act. 4.6 mCi/mmol), [^{14}C]carbaryl (naphthyl-1-labeled, spec. act. 10.23 mCi/mmol), [^{14}C]DDT (U-ring-labeled, spec. act. 29.7 mCi/mmol), and [^{14}C]dieldrin (methylene-bridge-labeled, spec. act. 85.0 mCi/mmol) were supplied by Amersham Corporation, Arlington Heights, IL; [^{14}C]nicotine (pyrrolidine-2-carbon labeled, spec. act. 54.01 mCi/mmol) was supplied by New England Nuclear, Boston, MA; [^{14}C]carbofuran (U-ring-labeled, spec. act. 2.85 mCi/mmol, and *cis*-[^{14}C]permethrin (benzyl alcohol labeled, spec. act. 56.0 mCi/mmol) were gifts from FMC Corporation, Middleport, NY, and [^{14}C]chlorpyrifos (2,6-ring-labeled, spec. act. 12.5 mCi/mmol) was a gift from Dow Chemical Company, Midland, MI.

Animals

Seven- to eight-weeks-old ICR strain female mice (25—30 g), were supplied by Flow Laboratories, Dublin, VA.

Surgical procedure

Mice were held without food 12 h prior to surgery, and water was provided ad lib. A longitudinal abdominal incision was made under ether anesthesia. The stomach was carefully exposed, and a ligature was secured at the pylorus. Care was taken not to damage or occlude major blood vessels. The stomach was returned to the normal location and the incision closed. Two hours were allowed for recovery prior to the treatment. Mice showing excessive bleeding or other adverse effects were discared.

Treatment

One tenth milliliter of carrier (Emulphor/ethanol/water, 1 : 1 : 8) was administered via an animal feeding needle (Popper and Sons, Inc., New Hyde Park, NY) as was done in a previous study [6]. Pesticide in the carrier and the fluid compartment of the stomach was found to be in solution (as previously reported [6]), which favored distribution in the stomach. The total dose of insecticide administered to each animal was 1 mg/kg including 1 μCi of labeled compound. No toxic symptoms were noted at this dosage level as reported in the previous study.

The treated mice were held in metabolism cages (Delmar) for the indicated time interval and then killed by ether. Bladder contents were combined with jar rinses. Blood was collected by heart puncture (0.5 ml), and the total radioactivity in blood was calculated assuming 77.8 ml blood/kg body wt [8]. The liver, stomach, and the remainder of the intestinal tract were

removed and the stomach was rinsed thoroughly with distilled water. The organs were homogenized separately from the remainder of the carcass; as amounts of radioactivity in kidney, fat, muscle, were found to be quite low in previous experiments at the early time intervals [6], these tissues were included with the carcass. Aliquots of the homogenates and of the stomach washings were oxidized in a Harvey Biological Oxidizer equipped with a CO_2 trapping device containing 15 ml of ^{14}C scintillation fluid (Harvey Instrument Corporation, Hillsdale, NJ). Radioactivity was determined in a Packard model 3330 liquid scintillation counter. Efficiencies were corrected based on the counting efficiency of an internal standard. Radioactivity in the stomach washing was used to estimate unabsorbed insecticide. All experiments were replicated 3 times.

Statistical analysis

The absorption data were analyzed using the logarithms of the percent absorption. Tests of differences among means in the log scale are equivalent to testing ratios of the absorption percentages.

Data were analyzed by Duncan's multiple range test and a 5% level of significance was implied [9]. To compare gastric to gastrointestinal absorption, ratios of the pooled mean square error were computed from the combined data sets from which a standard error (in log-units) of the mean differences was derived. To compare the several chemicals, the standard error was converted to a least significant ratio (LSR), such that any pair of gastric/gastrointestinal ratios differ significantly if their ratio exceeds the LSR.

RESULTS

The percentage of radioactivity disappearing from the ligated stomach contents after each time interval (as an indication of absorption) is shown in Table I. At the 15-min interval, carbaryl penetrated significantly faster than the other compounds, followed by carbofuran = dieldrin = chlorpyrifos < malathion = nicotine = permethrin and DDT in that general order although chlorpyrifos and malathion did not differ significantly from each other. At the 30-min period, carbaryl and carbofuran penetrated the most rapidly followed by dieldrin and chlorpyrifos, while malathion, DDT, nicotine and permethrin penetrated more slowly, although chlorpyrifos, malathion, and DDT did not differ significantly from each other. Sixty minutes after administration, the chemicals were grouped into the following categories regarding penetration: carbaryl, malathion, and carbofuran were the most rapid; dieldrin penetrated less rapidly; followed by chlorpyrifos; and a more slowly penetrating group included DDT, nicotine and permethrin.

With respect to distribution (Table II) it is noted that small amounts of radioactivity were recovered from all the organs investigated. The percentage of ^{14}C recovered (percentage of total absorbed dose) in liver and blood for all compounds studied was less than 1% except for carbaryl and carbofuran

TABLE I

ABSORPTION OF INTUBATED DOSES OF INSECTICIDES THROUGH THE LIGATED STOMACH OF MICE

Insecticide	% Absorption ligated stomach			% Absorption[a] gastrointestine (60 min)	Ratio, stomach/ gastrointestinal penetration
	15 min	30 min	60 min		
Carbaryl	14.1 A[b]	15.4 A	19.8 A	68.7	0.29
Carbofuran	9.5 B	15.1 A	18.8 A	67.1	0.28
Malathion	7.1 CD	8.1 CD	19.7 A	88.8	0.22
Chlorpyrifos	8.3 BC	9.5 BD	11.1 C	47.2	0.24
Dieldrin	9.5 B	11.5 B	13.9 B	63.2	0.22
DDT	5.3 E	7.5 CD	8.4 D	55.1	0.15
Nicotine	6.3 DE	6.7 D	8.2 D	82.9	0.10
Permethrin	6.2 DE	6.3 D	6.8 D	39.1	0.17
LSR[c]					1.41

[a] Data from Ahdaya et al. [6].
[b] Chemicals with the same letters do not differ significantly.
[c] LSR, least significant ratio.

which had a slightly greater amount at the 60-min interval. In the liver and blood, ^{14}C from carbaryl, carbofuran, malathion, and chlorpyrifos treatments was significantly higher than from dieldrin, DDT, nicotine, and permethrin. Dieldrin consistently showed the highest amounts associated with the stomach fraction. Only carbaryl- and carbofuran-treated animals showed appreciable amounts in the intestine whereas less than 1% of the other compounds were recovered. [^{14}C]carbaryl, -malathion, -carbofuran and -chlorpyrifos were found in measurable amounts in urine while only traces were found for other compounds. The ^{14}C recovered from the carcass reflects the trends seen for the other organs; that is, higher quantities were recovered for carbaryl, carbofuran, and malathion as compared to the other compounds.

DISCUSSION

It is evident from the results of this study that whereas all compounds under study showed appreciable rates of penetration from the ligated stomach, absorption was much slower than from the gastrointestine. Houston et al. [5] and Doluisio et al. [3] reported similar findings when comparing stomach and intestinal absorption in a group of drugs. To illustrate the contribution of gastric absorption to the overall process of gastrointestinal absorption, data from another study from this laboratory [6] were utilized to determine the ratio of stomach/gastrointestinal penetration (Table I). These values ranged from 0.29 for carbaryl to 0.1 for nicotine. Carbaryl has been reported to be absorbed very rapidly ($\simeq$80% in 60 min) from the

TABLE II

PERCENT ^{14}C RECOVERED (OF TOTAL ABSORBED DOSE) IN ORGANS AND TISSUES FOLLOWING ABSORPTION THROUGH THE LIGATED STOMACH OF MICE

Insecticide	Liver			Blood			Stomach[a]		
	15 min	30 min	60 min	15 min	30 min	60 min	15 min	30 min	60 min
Carbaryl	0.9 A[b]	0.5 AB	1.5 A	0.5 AB	0.7 A	1.2 AB	6.5 A	5.9 AB	4.4 BC
Carbofuran	0.4 ABC	0.7 A	0.9 AB	0.4 AB	0.6 A	1.2 A	2.8 C	4.1 B	4.0 BC
Malathion	0.7 AB	0.5 AB	0.8 AB	0.6 A	0.5 A	0.9 AB	0.1 D	0.5 D	5.4 B
Chlorpyrifos	0.3 BC	0.4 ABC	0.4 BC	0.3 B	0.5 A	0.5 BC	5.7 AB	5.8 AB	6.3 AB
Dieldrin	0.2 CD	0.3 BC	0.3 CD	0.1 CD	0.1 B	0.1 D	7.5 A	8.2 A	9.9 A
DDT	0.2 CD	0.1 E	0.2 CD	0.2 C	0.1 B	0.2 D	2.8 C	3.6 B	4.4 BC
Nicotine	0.1 D	0.2 CD	0.1 D	0.1 CD	0.3 A	0.1 D	3.9 BC	2.2 C	3.0 C
Permethrin	0.1 D	0.1 E	0.2 CD	0.1 CD	0.2 AB	0.4 C	3.2 C	3.8 B	3.7 BC
	Intestine			Urine			Carcass		
Carbaryl	2.7 A	3.4 A	5.6 A	0.2 BC	0.4 B	1.4 B	3.4 A	4.4 AB	5.6 A
Carbofuran	1.0 B	2.2 A	2.6 B	0.6 AB	2.7 A	5.3 A	3.8 A	4.7 A	4.9 A
Malathion	0.4 C	0.3 C	0.7 C	1.3 A	1.2 AB	5.3 A	4.2 A	5.2 A	6.6 A
Chlorpyrifos	0.2 CD	0.3 C	0.4 CD	Tr[c]	0.2 B	0.5 B	2.1 BC	2.3 BC	3.0 AB
Dieldrin	0.1 D	0.3 C	0.3 CD	Tr	Tr	Tr	1.7 C	2.6 BC	3.4 AB
DDT	0.2 D	0.4 BC	0.4 CD	Tr	Tr	Tr	2.0 BC	3.3 ABC	3.2 AB
Nicotine	0.1 D	0.7 B	0.2 D	0.1 BC	0.7 AB	0.7 B	2.0 BC	2.6 BC	5.2 A
Permethrin	0.1 D	0.3 C	0.4 CD	Tr	Tr	Tr	2.9 AB	2.0 C	2.0 B

[a] Quantity bound to the stomach was considered to be absorbed.
[b] Chemicals with the same letters do not differ significantly.
[c] Tr, $<0.1\%$.

ligated stomach of the rat [4], implying a difference between species. Nicotine was expected to show appreciably slower absorption in the slightly acidic stomach (pH 6.4 as determined from a measurement of 10 mouse stomach homogenates) as it would be highly ionized. As the majority of the insecticides showed 20% or greater absorption in the stomach, uptake by that organ could be of appreciable toxicological significance.

In all cases distribution was a reflection of absorption and was similar to a previous study on gastrointestinal absorption [6]. The compounds that showed higher penetration rates had more ^{14}C recovered from the organs. The higher amounts of carbaryl and carbofuran recovered from the intestine should be noted. Since the pylorus was ligated, the only explanation for the recovery of carbaryl and carbofuran would be through intestinal secretion. It has been previously reported that carbaryl shows intestinal secretion [4,6]. Metabolic fate of the compounds in distribution was assumed to be similar to a previous study [6].

Absorption from the stomach can be a significant route of entry for some insecticides. Approximately 25% of many insecticides may enter via the stomach during early stages of penetration. Even though nicotine showed only 10% entry via the gastric route due to ionization, this could be an important contribution to toxicity as has been previously suggested [1]. Stomach emptying could be a rate-limiting factor for some compounds, as those which are highly ionized, but would be expected to have less effect for such compounds as carbaryl, carbofuran, malathion, chlorpyrifos, and dieldrin. As appreciable amounts of chemical can be absorbed in the stomach, and conditions may favor stomach retention on occasions, gastric absorption can be an important toxicological parameter especially for the more toxic compounds. These studies expand this area of absorption of toxicants and confirm the conclusions of Karel [1] that gastric absorption may be greatly underestimated in toxicology.

ACKNOWLEDGEMENTS

This research was supported in part by PHS Grant No. ES-00044 from the National Institute of Environmental Health Sciences. Paper No. 7073 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC. The aid of Dr. R.J. Monroe in statistical interpretations is appreciated.

REFERENCES

1 L. Karel, Physiol. Rev., 28 (1948) 433.
2 L.S. Schanker, P.A. Shore, B.B. Brodie and C.A. Hogben, J. Pharmacol. Exp. Ther., 120 (1957) 528.
3 J.T. Doluisio, N.F. Billups, L.W. Dittert, E.T. Sugita and J.V. Swintosky, J. Pharmacol. Sci., 58 (1969) 1196.
4 H.H. Casper, J.C. Pekas and W.C. Dinusson, Pestic. Biochem. Physiol., 2 (1973) 3919.
5 J.B. Houston, D.C. Upshall and J.W. Bridges, J. Pharmacol. Exp. Ther., 195 (1975) 67.

6 S.M. Ahdaya, R.J. Monroe and F.E. Guthrie, Pestic. Biochem. Physiol., 16 (1981) 38.
7 J.C. Pekas, CRC Crit. Rev. Toxicol., 7 (1980) 37.
8 P.L. Altman and B.J. Dittmer, Biology Data Book, Fed. Am. Soc. Expt. Biol., Washington, D.C., 1964, p. 264.
9 R.G.D. Steele and J.H. Torrie, Principals and Procedures of Statistics, McGraw-Hill, NY, 1980, p. 187.
10 J.T. Doluisio, G.H. Tan, N.F. Billups and L. Diamond, J. Pharm. Sci., 58 (1969) 1200.