AN ABSTRACT OF THE THESIS OF

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Title: The In Vivo Metabolism of Ethyl-N, N-di-n-propylthiol-carbamate in Rats

Redacted for Privacy

Abstract approved S. C. Fang

The metabolism of ^{14}C-labeled ethyl-N, N-di-n-propylthiol-carbamate (EPTC-^{14}C) was studied in adult female rats. Single oral doses of EPTC-^{14}C ranging from 0.6 to 100.6 mg were given and the elimination pattern of ^{14}C was determined.

Results of 18 experiments showed that increasing the doses of EPTC-^{14}C led to a decrease in the ability of rats to catabolize the herbicide into respiratory carbon dioxide. The excretion through renal route, however, was enhanced. Thus, recovery of radioactivity in $^{14}CO_2$ decreased from 84.6% for the 0.6 mg dose to 38.2% for the 100.6 mg dose while recovery in the urine increased from 8.4% for the 0.6 mg dose to 35.6% for the 100.6 mg dose.

The rate of elimination of ^{14}C was markedly delayed by increasing the doses. $^{14}CO_2$ elimination was completed within 15 hours for 0.6 mg to 20.6 mg doses but extended to approximately 35 hours for 50.6 and a 100.6 mg dose. Likewise, the time

for complete elimination of radioactivity through urinary excretion was considerably extended. The pattern of $^{14}CO_2$ elimination showed a multiple peaked formation. This multiplicity of peaks was more pronounced with higher doses.

Paper chromatography and autoradiography revealed six major radioactive metabolites and three minor ones in the urine. Two dimensional thin layer chromatography and autoradiography, however, showed a total of 20 urinary radioactive metabolites, seven of which are the major ones. One of the major metabolites was tentatively identified as urea.

Attempts to isolate the individual metabolites through ion exchange chromatography or extraction with organic solvents has not been successful. These procedures, as well as elution of metabolites from the paper chromatograms, demonstrated the labile nature of some metabolites.

The *In Vivo* Metabolism of Ethyl-N, N, -di-n-propylthiolcarbamate in Rats

by

Visitacion Yu Ong

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Associate Professor of Chemistry
in charge of major

Redacted for Privacy

Chairman of Department of Biochemistry and Biophysics

Redacted for Privacy

Dean of Graduate School

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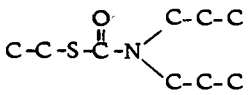
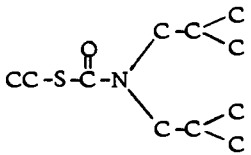
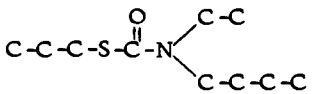
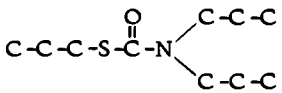
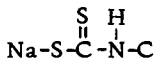
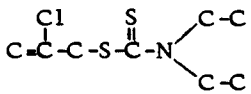
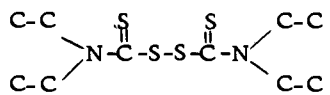
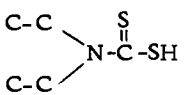
INTRODUCTION

The thiol- and dithiocarbamates have found extensive application as pre- and postemergence herbicides on field and vegetable crops, and forage legumes. A list of these sulfur containing herbicides is given in Table 1. Ethyl-N, N, -di-n-propylthiolcarbamate (EPTC) was the first of these thiolcarbamates introduced by Stauffer Chemical Company in 1954 for effective pre-emergence control of broad-leaf weeds and all annual grasses growing from seeds. The mode of action and fate of EPTC, or thiolcarbamates as a whole, is still little known, although carbamate herbicides are recognized as mitotic poisons that exert their lethal action in root meristems when used as soil-borne herbicides.

Studies of EPTC in recent years have been focused on the investigation of its physical properties in relation to its behavior and persistency in soil (Danielson, Gentner, and Jansen, 1961; Fang and Freed, 1961; Havis, Trichnor, and Bobula, 1959). Absorption and translocation by resistant and susceptible plants had also been investigated by a number of workers (Crafts, 1959; Fang and Theisen, 1960; Yamaguchi, 1961).

Relatively few studies has been reported on the metabolic fate of thiolcarbamates in the biological system. The possible sites of

Table 1. Chemical Structure, Chemical Name, and Common Name or Designation of Thiol- and Dithiocarbamates

Chemical Structure	Chemical Name	Common Name or Designation
	Ethyl N, N-di-n-propylthiolcarbamate	EPTC (Eptam)
	Ethyl N, N-diisobutylthiolcarbamate	R-1910
	S-Propyl butylethylthiocarbamate	Perbulate (Tillam)
	S-Propyl dipropylthiocarbamate	Vernolate
	Sodium N-methyldithiocarbamate	SMDC
	2-Chloroallyl diethyldithiocarbamate	CDEC
	Tetraethylthiuramdisulfide	Disulfiram (Antabuse)
	Diethyldithiocarbamate	

attack are the alkyl groups, the amide linkage, or the ester linkage. The initial site of attack is to a great extent determined by the size of the alkyl groups attached to the carbamate linkage (Kaufman, 1967). In the presence of relatively small alkyl groups, the thiolcarbamate molecule is probably hydrolyzed at the ester linkage.

A study on the degradation of EPTC-^{35}S in germinating seed was carried out by Fang and Yu (1959). They reported that the radioactivity in seeds of resistant plants exposed to EPTC-^{35}S decreased with time, ^{35}S label was incorporated into sulfur amino acids cystine, cysteic acid, methionine, and methionine sulfone. However, a study with ethyl-1-^{14}C-labeled EPTC of the fate of this herbicide in the alfalfa plant (Nalewaja, 1966) revealed no incorporation of radioactivity into sulfur amino acids. Instead ^{14}C was found to be incorporated into glucose, fructose, and several other amino acids. This finding, coupled with the earlier results of Fang and Yu, suggest a further cleavage between the sulfur atom and the ethyl group, following an initial attack at the ester linkage of EPTC. Their results ruled out the direct incorporation of ethyl mercaptan into sulfur amino acids.

To date the only study reported of the metabolism of thiolcarbamate herbicide in mammals is that of Fang, George, and Freed (1964) on the metabolism of tillam in rats. They indicated that tillam was rapidly metabolized and eliminated from rats after oral dosing.

Almost all radioactivity was recovered in two days in expired $^{14}CO_2$, urine, and feces. Recovery in $^{14}CO_2$ accounted for 55% of the dose and 23% was excreted in the urine. The detection of 20 to 22 urinary radioactive metabolites points to the extensive breakdown and biotransformation of tillam. Since some of the identified urinary products--amino acids as well as urea--contained label from tillam molecule, the authors postulated that the thiolcarbamate must have been attacked at the ester linkage to give the corresponding mercaptan, alkylamine, and CO_2. Transthiolation of the mercaptan may give rise to an alcohol which was further oxidized to an acid prior to entering the metabolic pool. The metabolic scheme is presented in Figure 1. This scheme agrees with the results obtained from plants.

Inasmuch as the current literature concerning the metabolism of thiolcarbamate is scarce, it will be relevent here to review briefly the general process involved in detoxification of foreign compounds and consider some of the information resulting from the investigations of the related carbamates.

Metabolic transformation of xenobiotics--compounds foreign to life--can be classified as nonsynthetic and synthetic. The nonsynthetic reactions involve oxidation, reduction, and hydrolysis. The synthetic reaction, or conjugation, involves the coupling between the foreign compound or its metabolite and an endogenous substrate such as glucuronic acid, mercapturic acid, sulfate and various

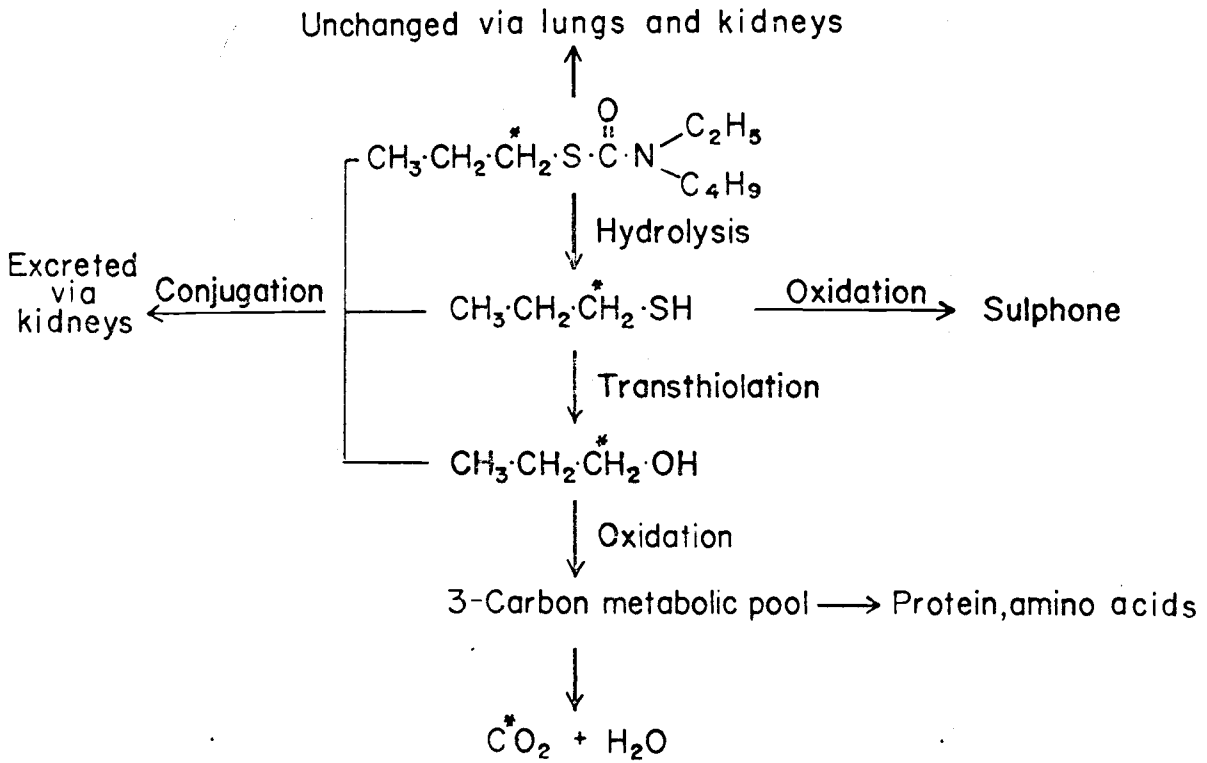
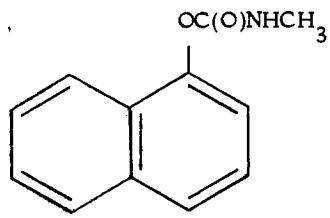


Figure 1. Proposed metabolic pathways of Tillam-^{14}C in rats.

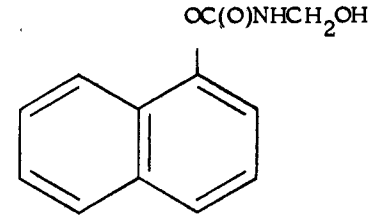
amino acids. As a result of conjugation, a more water soluble and highly ionized compound is formed which can be readily excreted in the urine. Therefore conjugation reaction almost invariably led to the inactivation of the foreign compound.

Oxidation reactions include O- and N-dealkylation, alkyl and aryl hydroxylation, sulfoxide formation, deamination, and epoxidation. These reactions are catalyzed by oxidation enzymes and thus require NADPH and O_2. Since reduction processes are catalyzed by flavoprotein, NADPH is also needed as H-donor. Conjugation, on the other hand, is an endothermic reaction that does not require NADPH. ATP is required as an energy source. All enzymes involved in metabolic transformation are located in liver microsomes. It is therefore not surprising that metabolism of xenobiotics occurs mainly in the liver.

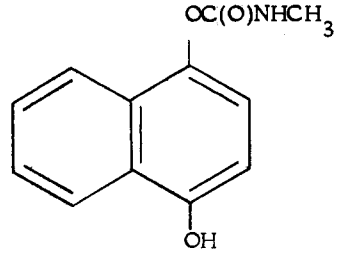
The most extensively studied of carbamates is carbaryl (1-naphthyl-N-methylcarbamate). Several in vitro studies using liver microsomes with added NADPH revealed the formation of hydroxylated organosoluble metabolites having the ester linkages intact (Dorough and Casida, 1964; Knaak et al., 1965; Hassan et al., 1966; Leeling and Casida, 1966). The structure of the three identified major hydroxylated metabolites and the parent compound carbaryl were given below:



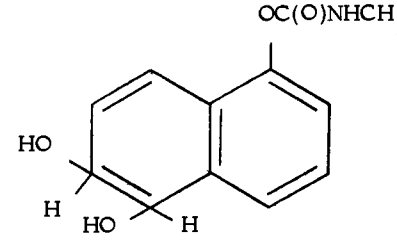
1-Naphthyl-N-methyl-carbamate (Carbaryl)



1-Naphthyl hydroxyl-methylcarbamate



4-Hydroxy-1-naphthyl methylcarbamate

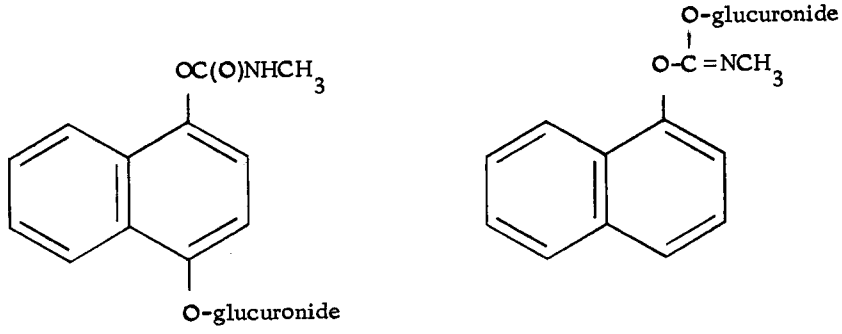


5,6-Dihydro-5,6-dihydroxyl-1-naphthyl methylcarbamate

The first detailed investigation on the carbaryl metabolism in the rat, the guinea pig, and man was reported by Knaak et al. (1965). The over all recovery of radioactivity from methyl-^{14}C, carbonyl-^{14}C, and naphthyl-^{14}C carbaryl given to rat and guinea pig was 95, 99 and 91%, respectively, of the dose. Only 2 to 3% of the label from carbaryl-methyl-^{14}C was detected in the tissue after 7 days. This study showed that a nonhydrolytic pathway exists for carbaryl, thus 47-54% of the metabolites excreted possess the intact C-O-C(O)N-C structure.

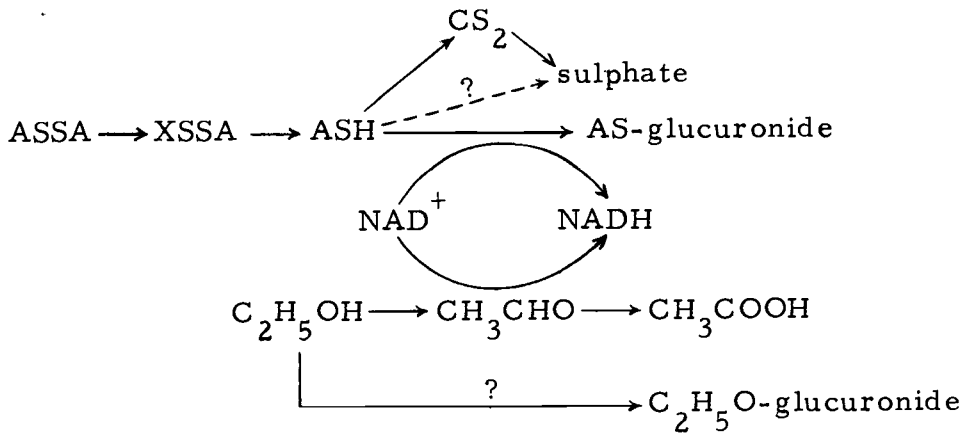
Hydrolytic pathway led to the liberation of naphthol and respiratory $^{14}CO_2$. The liberated naphthol was found to be excreted as conjugate of sulfate and glucuronic acid.

Distribution of urinary metabolites differed slightly among species studied. They found at least seven in the rat, nine in the guinea pig and six in man. The metabolites identified and tentatively identified in the urine of rat and guinea pig were glucuronide of 4-hydroxylcarbaryl, and O-glucuronide of the enol form of carbaryl respectively.



Hydrolytic removal of $OC(O)NHCH_3$ group was also shown to be a major pathway of the metabolism of several other carbamates in rats (Krishna, 1966). Both in vitro and in vivo, the hydrolytic product 1-naphthol undergo conjugation with glucuronide and sulfate. With N-dimethylcarbamate, the major pathway of metabolic breakdown was demonstrated to be side chain hydroxylation (Hodgson and Casida, 1960; Hodgson and Casida, 1961).

In vivo studies of the fungicide disulfiram (antabuse) in rats had demonstrated its immediate conversion into parent thiol diethyldithiocarbamate (Strömme, 1965). This thiol was further metabolized at a high rate through several pathways: (1) Conjugation with glucuronic acid to form S-glucuronide; (2) oxidation to sulphate; (3) decomposition to carbon disulphide and diethylamine; (4) reoxidation to disulfiram. Free disulfiram was never detected in plasma, liver or in urine. Reduction to parent thiol was attributed to the glutathione-gluthathione reductase system of metabolizing erythrocytes. A scheme was presented for the catabolism of disulfiram and diethyldithiocarbamate in the rat along with the steps of the ethanol metabolism which the author considers to be relevant to the ethanol-induced inhibition of the glucuronic acid conjugation.



ASSA (disulfiram)
ASH (diethyldithiocarbamate)

The formation of S-glucuronide was demonstrated to play an important role in the metabolism of disulfiram.

It is known that the metabolism of xenobiotics can result in the formation of either nontoxic substances or metabolites that are more toxic than the parent compound. Although the study with resistant plants showed that EPTC is relatively non-persistent, a direct study on its fate on ingestion by mammals is of major significance in establishing safe limits for residue in foods and feeds. It is hoped that the present study will shed more light on the biochemistry of the detoxification of this thiol compound. Included in this study will be:

(1) Observation of rate and pattern of ^{14}C elimination, and the influence exerted by increasing the doses.

(2) Determinination of the accumulation of radioactivity in various organs.

(3) Analysis and preliminary examination of the metabolites in the excreta.

MATERIALS

The female rats used for this study were of an inbred Oregon State Wistar strain, aged seven months to one year, and weighed 233 to 290 grams.

The herbicide ethyl-1-^{14}C(N-di-n-propyl)thiolcarbamate (1.33mc/mM) was obtained from Research Specialties Co., Richmond, California. The stock solution was prepared by dissolving the radioactive EPTC in 95% ethyl alcohol to give a final concentration of 29.0 mg per ml. For oral administration, aliquot of this stock solution was diluted with water to give the desired concentration. Higher concentration of EPTC was prepared by dissolving the unlabeled EPTC in Wesson oil to give concentrations of 48.3 mg per ml and 96.6 mg per ml. A known amount of the unlabeled EPTC was then administered along with labeled EPTC. Non-labeled EPTC was obtained from Stauffer Chemical Company, Richmond, California.

The counting medium used for radioanalysis with liquid scintillation spectrometer consisted of 5 ml of each of the following solutions: A. Toluene phosphor solution: 4 grams of 2,5-diphenyloxazol (PPO), and 50 mg of 1,4-bis-2-(5-phenyloxazol) benzene (POPOP) per liter of toluene. B. Methylcellosolve-naphthalene solution: 50 grams of naphthalene dissolved in a liter of methylcellosolve. For counting with non-quenching organic solvent

only solution A was used. Benzoic-7-^{14}C acid dissolved in toluene was used for internal standardization.

METHODS

Administration of Chemicals

EPTC-^{14}C was administered orally to the rats by the use of a syringe and a thin Teflon tubing 0.022 inches in diameter and 3.5 inches long. The rats were lightly anaesthetized with diethyl ether vapor before dosing. They were placed immediately in the Delmar glass metabolism cages after dosing.

Collection of Urine, Feces and $^{14}CO_2$

The use of Delmar glass metabolism cage permitted the separation and simultaneous collection of urine, feces, and $^{14}CO_2$. Air was pumped into the cage at a rate of 426 cc per minute and was checked constantly with Matheson Universal Flow meter model 203. The exhaled $^{14}CO_2$ was allowed to pass through a Drierite column to absorb moisture before entering the flow ion chamber which was connected to an electrometer. Two rats were used for each run; thus two electrometers were used, one, a Cary model 3810 with 500 cc ionization chamber, and the other, a Dynacon model 6000 with the same capacity. The $^{14}CO_2$ output was monitored continuously with recorders and the chart speed was set for one inch per hour for Cary and 3/4 inch per hour for Dynacon. The experiments were terminated

after 72 hours for lower doses. Due to the slower rate of radioactivity elimination, the length of each run was extended to 96 hours for 50.6 and 100.6 mg doses.

Estimation of $^{14}CO_2$ expired was made by determining the total area under the $^{14}CO_2$ evolution pattern and extrapolation from the $^{14}CO_2$ standard curve obtained from known quantities of $CH_3{}^{14}COONa$ given to the rats. The curve is shown in Figure 2.

Before each experiment, a few drops of toluene were added to the separator trap to act as a preservative of urine samples. Both urine and feces were collected every 24 hours, and the separator traps rinsed thoroughly with 50% ethyl alcohol. The wash was added to the original urine sample. In cases where feces were contaminated with urine, a disposal dropper was used to draw out the urine as much as possible and to combine it with the urine sample. Food particles in the urine were removed by centrifugation.

Aliquots of the urine were taken for radioanalysis using a Packard Tricarb Liquid Scintillation Spectrometer model 314EX-2. The bulk of the remaining sample was either freeze dried or frozen for further analysis.

Feces were chopped up and extracted repeatedly with 50% ethyl alcohol until all activity had been extracted. The extract was separated from the residue by centrifugation. Aliquots of the extract were taken for radioactivity measurement by liquid scintillation. The residue was dried by air jet, ground with mortar and pestle to

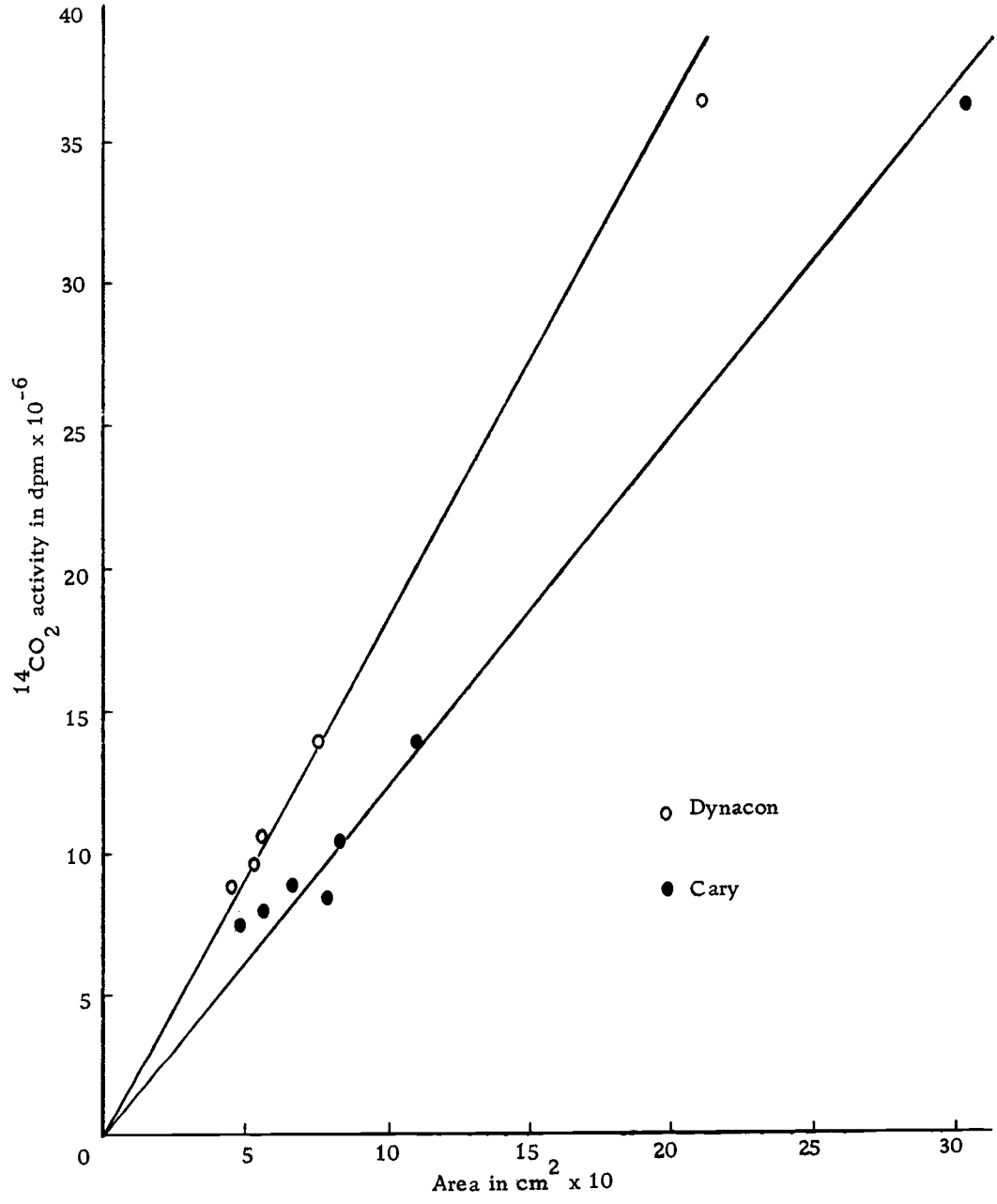


Figure 2. Standard curve for conversion of voltage response to $^{14}CO_2$.

Full scale: 100 mv
Air flow rate: 426 ml/min
Chart speed: Cary: 1"/hr.
Dynacon: 3/4"/hr.

fine powder, weighed into alumina planchet, and the activity determined with a Geiger-Mueller counter. All counts were corrected for background and self absorption.

Laboratory Purina Chow and drinking water were available to the rats at all times.

Analysis of the Urine

Determination of Residual EPTC-^{14}C in the Urine

The extraction procedure for EPTC as outlined by Stauffer Chemical Co. and modified by Fang and Theisen (1959) was used in this experiment. Steam distillation was carried out in volatile oil lighter than water set up. The moisture trap was filled with water to the zero mark and 5 ml of isooctane was introduced into the column. A known quantity of urine was diluted with water in a round bottom flask. This was heated in an electric mantle and allowed to boil for one hour. During this period, the steam condensate was continuously extracted with isooctane, which was drawn out through the stopcock at the end of one hour. The total volume of isooctane was noted, aliquot of the extract was used for radioactivity measurement. The total radioactivity recovered in the isooctane extract represents the quantity of the residual EPTC-^{14}C that might be present in the urine.

Paper Chromatography

Radioactive metabolites in the urine were separated by paper chromatography. An aliquot of urine sample was applied as a band on 1×18 1/2" Whatman #1 chromatography paper strips 2 3/4" from the end of the strips. The strips were developed one dimensionally at room temperature by the descending method. Four solvent systems were used for comparison: BAW (n-butanol:acetic acid: water, 8:2:3, v/v/v); PEW (propyl alcohol:ethyl acetate:water, 6:1:3, v/v/v); EAW (95% ethyl alcohol:ammonia:water, 18:1:1, v/v/v) and saturated phenol (500 gm phenol in 125 ml H_2O). All paper strips were allowed to equilibrate with the solvent for at least half an hour in the developing chamber before the solvent was introduced. The solvent was allowed to run to approximately two inches from the other end of the strips which required 12 to 18 hours depending on the type of solvent. After air drying, the paper strips were scanned by the Radiochromatogram Scanner Packard model 7200 to determine the number of radioactive metabolites present in the urine sample. The percentage distribution of each metabolite and their R_f value were calculated.

Colorimetric Tests on Paper Chromatograms

Preliminary attempts were made to determine the nature of radioactive metabolites in the urine by color reagents for amino

acids, amine, carbohydrates and sulfur compounds. These color reagents were applied to the dried, scanned chromatograms either as spray or dip. After the development of color, the strips were compared with the original scanning records.

Amino acids and amine: A 0.25% solution of ninhydrin in 95% ethyl alcohol was used. The color was allowed to develop at room temperature. After maximum color development with ninhydrin, the paper strips were scanned at 1 mm intervals along the length of the chromatogram using Photoelectric Densitometer (Photovolt Incorporation). The amount of transmittance recorded on the galvanometer gave approximately the color density of each spot.

Carbohydrates. Carbohydrates were detected by the use of diphenylamine - aniline - phosphoric acid reagent (DAPA) of Kocourek, Ticha, and Kostir (1966). Maximum color development was obtained after the strips were warmed in the oven at 110° for 3 minutes.

Sulfate. The method of Knight and Young (1958) for the detection of compounds containing bivalent sulfur was employed. The dried chromatograms were sprayed first with 0.1 M $K_2Cr_2O_7$ - acetic acid (1:1), and then with 0.1 M $AgNO_3$.

Sulfoxides, sulfones and sulfides. Snow (1957) and later Lowe (1959) had investigated the metabolism of compound related to ethyl mercaptan in the guinea pig. One of the major urinary metabolites identified in both studies is ethyl methyl sulphone. A color test on

papers was therefore carried out to detect the presence of sulfur compounds in any of these oxidation states. Two detecting reagents were used according to Fishbein and Fawkes (1966).

(1) Chloranil: 1% tetrachloro-p-benzoquinone in benzene.

(2) Gibbs reagent: 2% 2, 6-dibromo-N-chloro-p-benzoquinoneimine in benzene.

Both reagents were applied as spray. The sprayed paper was then passed over ammonia vapor where the color of the spot changed accordingly or intensified. These reagents were reported to give clear distinction of sulfoxides, sulfones and sulfides.

Urea. If metabolic breakdown of EPTC follows the scheme proposed for tillam, acetate would be expected to be one of the intermediate which would give rise ultimately to urea as one of its end products in the urine (Philleo and Fang, 1968). To establish this, the urine sample was co-chromatographed with urea standard using different solvent systems. Urea was identified with Ehrlich reagent, a solution containing equal volume of p-dimethylaminobenzaldehyde and concentrated hydrochloric acid. One part of this solution was mixed with four parts of acetone just before use (Smith, 1960).

Uronic acids. Naphthoresorcinol reagent was used for the detection of uronic acid conjugation products. 0.2% of naphthoresorcinol in acetone (5 vol.) was mixed with 9% phosphoric acid (1 vol.). The paper was dipped in the mixture, and placed in

the oven at 95°C for 5-7 minutes (Smith, 1960). This method was used successfully for the detection of β-ethyl-D-glucuronide (Kamil, Smith, and Williams, 1952).

Thin Layer Chromatography

Since EPTC is volatile, difficulty was encountered for its detection on the paper chromatogram. Consequently metabolites that may contain the whole molecule of EPTC may have escaped detection. A technique used for detecting and partially characterizing carbamate and thiolcarbamate at μg levels after on-plate (thin layer plate) hydrolysis to amines had been described (Askew, Ruzicka, and Wheals, 1968). The specific color reaction aid in the characterization of the parent pesticides or herbicides.

The thin layer plates were prepared according to Waldi (1965). The plates (5 × 20 cm) were coated with 250μ layer of Silica Gel G. They were activated at 110° C for 30 minutes before use. Standard EPTC-^{14}C was then spotted on one corner of the plate and the urine sample spotted on the other corner. After developing in suitable solvent system in the cold (0°C), the plate was dried and scanned with the use of a radiochromatogram scanner. Following scanning, the plate was sprayed with hydriodic acid. Another glass plate was then clipped over the sprayed surface and the whole heated in an oven at 180°C for 30 minutes. The plate was

removed after 30 minutes and allowed to cool. It was sprayed with ninhydrin and heated in an oven for 20 minutes at 120°C.

The solvent systems tested on different plates were:

I. BAW (n-butanol:acetic acid:water, 8:2:3, v/v/v).
II. Isopropanol:formic acid (6N):water (40:2:10, v/v/v).
III. Benzene:ethanol:water:ammonia (150:47:15:1), upper phase.
IV. Butanol:acetone:water (4:5:1).

The information obtained with radioscanning of one dimensional thin layer plates was used in carrying out two dimensional chromatography with 20 × 20 cm plates. BAW was used as the first solvent, and either solvent II, III, or IV was used as the second solvent. Commercial thin layer ChromAR sheet 500 from Mellinckrodt was also employed. All two dimensional chromatograms were then subjected to autoradiography prior to spraying with coloring reagents.

Autoradiography

Determination of radioactive metabolites by the use of the radiochromatogram scanner is usually less sensitive. Low activity peaks may be overlooked and the shapes of radioactive spots may be difficult to determine. Thus autoradiography of the one dimensional paper chromatogram and two dimensional thin layer chromatograms was carried out.

Paper and thin layer chromatograms of urine sample were

exposed to 11 × 14" Eastman Kodak Medical X-ray film for a period of at least one month. After the exposure period, the films were developed with Kodak D-19 and fixer at room temperature, rinsed with running water for two to three hours and air dried.

Separation of Radioactive Urinary Metabolites

To facilitate the analysis of urinary metabolites, attempts were made either to separate the individual metabolites out or to separate them into smaller groups for further purification. Three methods were examined: solvent extraction of freeze dried urine sample, ion exchange column chromatography, and elution of the metabolite from paper chromatogram after separation.

Solvent Extraction. The freeze dried urine was extracted repeatedly using non-polar to more polar solvents. Three organic solvents used were benzene, ethyl acetate, and ethyl alcohol, in the sequence mentioned. Extractions were carried out in the following manner: five ml fractions of the solvent were introduced into the vials containing known activity of freeze dried urine. The caps were replaced tightly and the contents were shaken occassionally. The vials were allowed to stand overnight after which the solvents were decanted, and aliquots were taken for radioactivity measurement. Extraction was repeated with fresh 5 ml fractions of the same solvent until no activity was detected. The residues were then freeze dried,

the new solvents were introduced and the process was repeated.

For paper chromatography, the first 5 ml fraction of the extract of each solvent was used, since this was the fraction with the highest activity.

Ion exchange chromatography. The use of column chromatography in this experiment was mainly to separate the urinary metabolites into cationic, anionic, and neutral fractions. Therefore no pH adjustment was made during the operation.

Freeze dried urine was taken up with a small amount of distilled water and placed on a column (a 5 ml pipet packed with Dowex 50W×12 in the hydrogen ion form). The column was washed with distilled water and 5-10 ml fractions were collected until the activity in the last fraction decreased to that of the background. All fractions were combined and freeze dried. This was taken up with a small amount of water and introduced into the anionic column (a 5 ml pipet packed with Dowex 2-X8 in Cl^- form). Again the column was washed with distilled water, fractions collected, and activity of each fraction determined. The elution of metabolites from the anionic column was achieved by the use of 1 N HCl. No elution was carried out with the cationic column since all activity was recovered in the water wash after the sample passed through the cationic column.

The neutral fractions (the wash), and the anionic fractions (the HCl eluate), were freeze dried to reduce the bulk and concentrate

the activity. It was taken up with a few drops of water for paper chromatography.

Elution of Urinary Metabolites from Paper Chromatograms. For elution, the 3MM Whatman filter papers werecut into 18" long strips and washed with developing solvent BAW. After the papers had been washed and dried, urine samples from rats treated with labeled and unlabeled EPTC were applied to the papers separately. As much sample was applied as possible. The strips were developed as before with BAW. Using the chromatogram scannings of the radioactive EPTC treated urine as a guide, the areas corresponding to the respective metabolic peaks of non-labeled chromatogram strips were cut out. These small pieces (one or two at a time) were placed between a folded strip of filter paper and two microscope sides. Both ends of the paper strips projected out of the edge of the glass slides. When the glass slides were fastened to the small beaker by a short string, one end of the strip could be folded back into the beaker containing the solvent and the other end of the strip,from which the eluent dropped into the receiving beaker, could hang free. When the set-ups were ready, a glass lid was placed over the assemblage to help maintain the saturated solvent vapor. The elution was carried out both at room temperature and at cold room (0°C).

Elution was allowed to continue until all the compound in the paper had been eluted. The volume of the eluate and the time required

for complete elution were determined the first time by eluting the radioactive sample and checking the eluate activity constantly. The papers were likewise checked when the elutions were deemed complete.

Analysis of the Internal Organs for Radioactivity Accumulation

In six separate runs, all rats treated with 3 mg, 53 mg, and 103 mg of EPTC-^{14}C were sacrificed 26 hours after dosing.

All internal organs were removed promptly and freeze dried. They were then ground into fine powder. Their activities were determined by a Geiger Mueller counter.

RESULTS AND DISCUSSIONS

Elimination Pattern of $^{14}CO_2$ after Oral Administration of EPTC-^{14}C

EPTC-^{14}C was rapidly metabolized and eliminated from rats after single oral dosing. Elimination of $^{14}CO_2$ from rats dosed with 0.6 mg to 20.6 mg of EPTC was completed within the first 15 hour period. When the dose was increased to 50.6 and 100.6 mg, the time required for complete elimination was lengthened to 30-35 hours. This is illustrated in the continuous scanning recording of respiratory $^{14}CO_2$ in Figure 3. All rats survived the experimental period without ill effect even as the dose was increased to 100.6 mg.

In all 18 experiments, the first peak appeared within 1-2 hours after dosing. This peak in most cases represented the maximum peak of $^{14}CO_2$ elimination. However, the decrease in radioactivity did not follow a regular hourly declining pattern. Metabolic breakdown of EPTC produced instead multiple $^{14}CO_2$ peaks (Figure 4). This multiple peaked and erratic pattern was more pronounced with higher doses.

The percentage of recovery of $^{14}CO_2$ was inversely proportional to the doses. Increasing doses led to decreasing recovery in $^{14}CO_2$. Tabulated results in Table 2 showed that recovery as $^{14}CO_2$ accounted for 84.6% of the radioactivity for the 0.6 mg dose

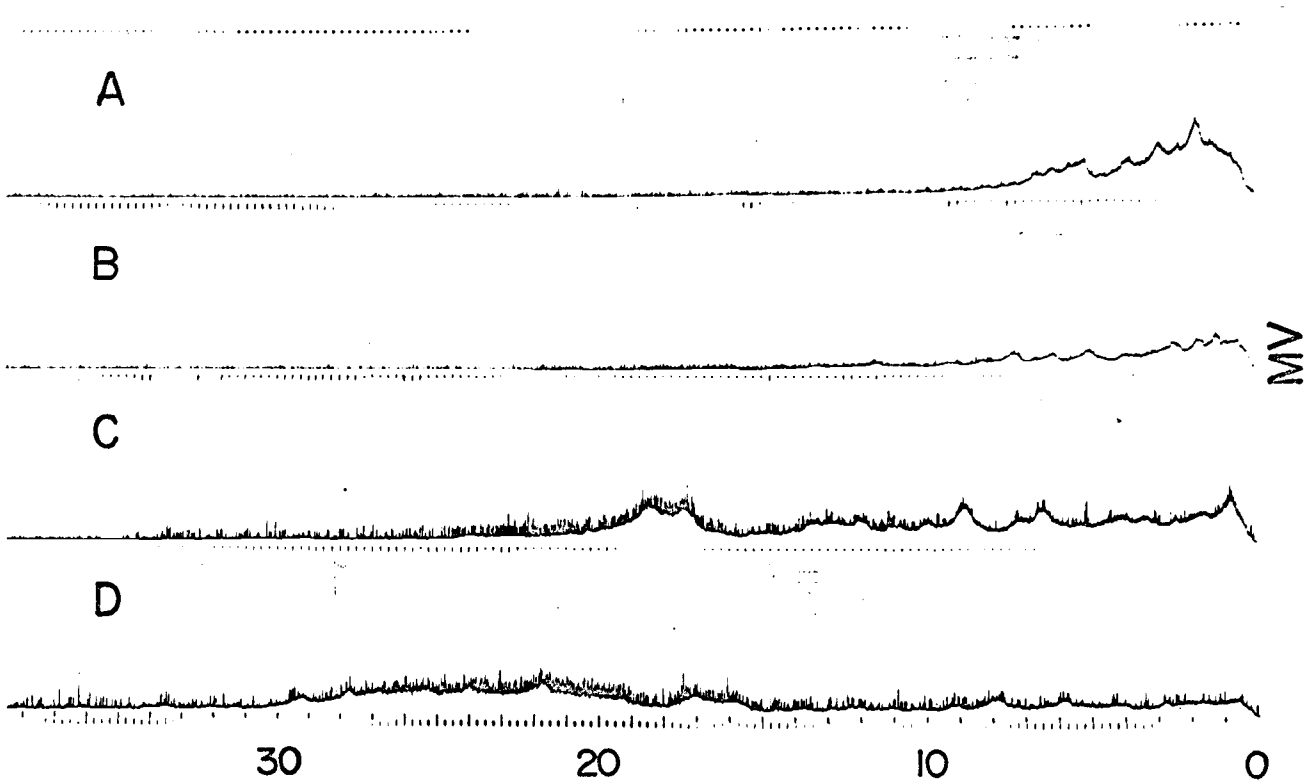


Figure 3. Elimination pattern of expired $^{14}CO_2$ as recorded by a vibrating-reed electrometer (Cary model 3810).

A. 0.6 mg, 100 mv
B. 20.6 mg, 100 mv
C. 50.6 mg, 30 mv
D. 100.6 mg, 30 mv

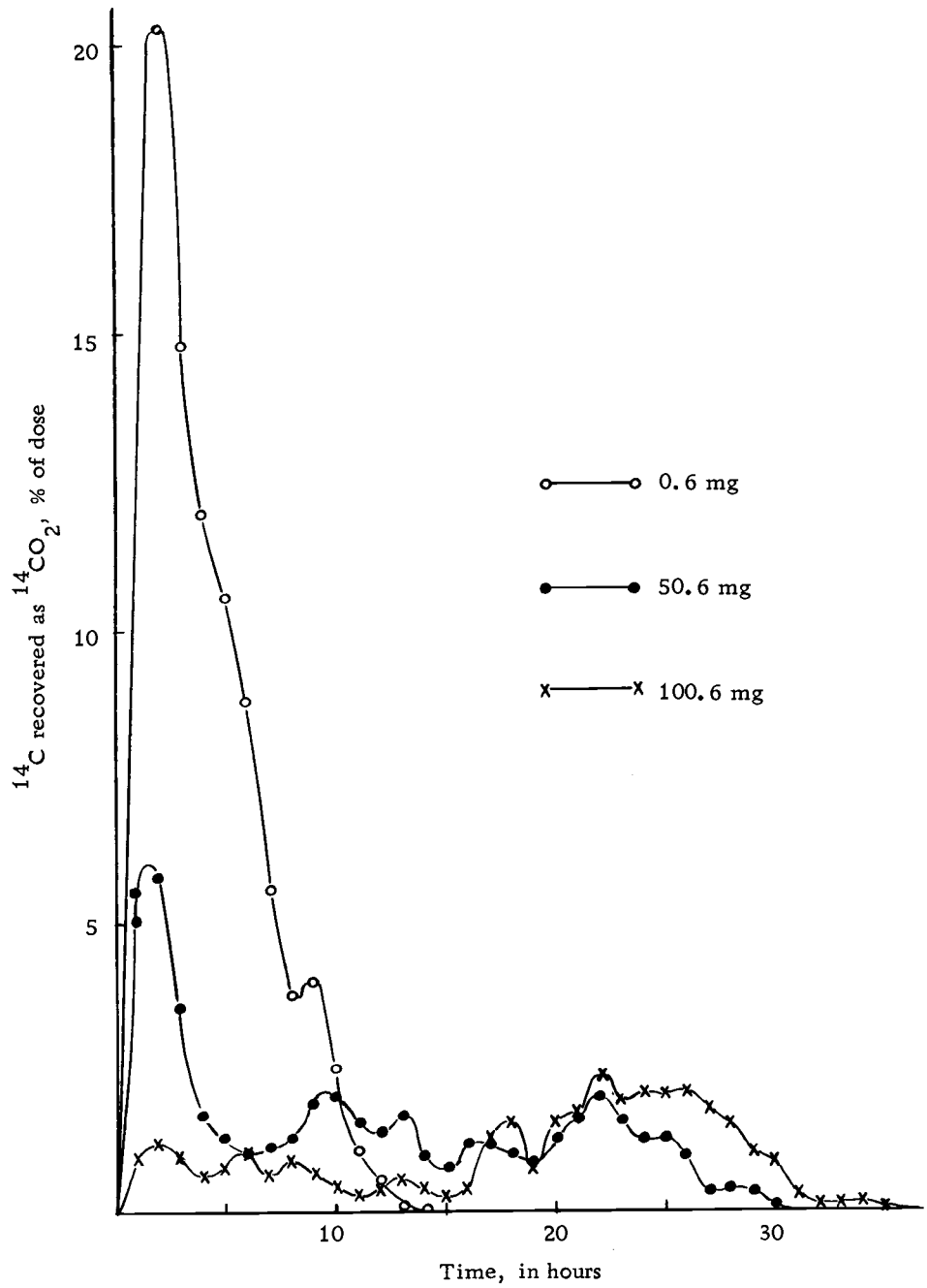


Figure 4. The effect of increasing doses on the hourly recovery and elimination pattern of expired $^{14}CO_2$ from treated adult female rats.

Table 2. Total Recovery of Radioactivity from Excreta of Rats Treated with Varying Doses of EPTC-^{14}C in 96 Hours

	Percent of dose recovered in 96 hours			
Dose (mg)	Urine	Feces	$^{14}CO_2$	Total
0.6 (4)	8.4	4.4	84.6	97.4
1.0 (3)	8.0	11.6	69.8	89.4
1.5 (3)	11.6	4.0	72.1	87.7
3.0 (2)	16.9	4.7	64.4	86.0
20.6 (2)	16.6	7.0	60.6	84.1
50.6 (2)	17.4	12.6	47.6	77.6
100.6 (2)	35.6	11.0	38.2	87.8

( ) = number of run

as compared with 38.2% for the 100.6 mg dose. The length of time required for the complete elimination of the higher doses of EPTC, as was mentioned before, was considerably prolonged. The ability of the enzyme systems involved in catabolism to $^{14}CO_2$ was obviously limited and thus the herbicide is oxidized only to a certain extent. No in vitro studies with any of the enzymic system were carried out.

From a toxicological point of view, the extended duration of the radioactivity in the physiological system with higher doses is undesirable and leads ultimately to a problem of residue accumulation.

Since EPTC is lipid soluble, it is expected to be readily absorbed through the gastrointestinal tract and transported into the liver, where most of the detoxification processes take place. Therefore the rate of absorption of EPTC through the gastrointestinal tract seems unlikely to be the factor affecting the rate of elimination of $^{14}CO_2$. The multiple peaks in the $^{14}CO_2$ pattern is rather suggestive of a diversified pathway of elimination.

If EPTC is hydrolyzed at the ester linkage, as the experimental results here seem to indicate, the resulting products will be ethyl mercaptan, dipropylamine, and CO_2. Studies by Lowe (1959) of the metabolism of compounds related to ethyl mercaptan in guinea pigs revealed that 50% of the administered ^{14}C appeared as $^{14}CO_2$. This result agreed with the finding of Canellakis and Tarver (1953), who

studied the metabolism of methyl mercaptan in rats and reported about 10% recovery in $^{14}CO_2$ in six hours. Lowe (1959) suggested that the action of desulphydrase upon ethyl mercaptan removed hydrogen sulphide and left a two carbon unit. Subsequent oxidation of hydrogen sulphide and metabolism of a two carbon unit would lead to the equivalent production of CO_2 and SO_4. This scheme seems plausible in view of the fact that desulphydrases have been found in higher animals (Fromageot, 1951), and oxidation of hydrogen sulphide to sulphate has been demonstrated in the dog (Dennis and Reed, 1927) and rats (Dziewiatkowski, 1945).

Since the major portion of activity in this study was recovered in $^{14}CO_2$, the transthiolation of ethyl mercaptan to ethyl alcohol must necessarily be the major pathway involved in the degradation of EPTC-^{14}C. The problem then resolved into the metabolism of ethyl alcohol, which is well documented. Substantial evidences indicate that the metabolism involves an initial oxidation to acetaldehyde, a conversion of the latter to acetyl co-enzyme A (CoA) either directly or via acetic acid, and the complete combustion of acetyl CoA in the citric acid cycle. Thus ethyl alcohol labeled in both carbon has been determined to be largely oxidized to CO_2 (approximately 90% of the dose) (Bartlett and Barnet, 1949). The possibility that an alternate pathway for the metabolism of ethyl alcohol exists is due to the following findings: (1) the better utilization

of ethanol, for the synthesis of cholesterol, fatty acids and acetyl sulfanilamide, as compared with acetate (Synder, Schulman, and Westerfeld, 1964), (2) the different rate at which each carbon of ethanol and acetate is converted to CO_2 (Russell and Bruggen, 1964), (3) the presence of an unidentified metabolite in tissue homogenate from labeled ethanol, but not from acetate (Westerfeld and Schulman, 1959). Russell and Bruggen suggested that the difference in utilization and rate of conversion to CO_2 between ethanol and acetate may be related to the relative permeabilities of membranes to ethanol and acetate, the relative endogeneous pool sizes for these two compounds, and their respective activation. Either ethanol is converted directly to acetyl CoA or via acetic acid, the rate of conversion to CO_2 probably differed which might explain, in part, the appearance of multiple peaks.

A new metabolite, 5-hydroxy-4-ketohexanoic acid, was identified after incubation of rat liver or kidney homogenates with ethanol-1-^{14}C (Bloom and Westerfeld, 1966). This metabolite was thought to be formed by a decarboxylation condensation reaction between acetaldehyde and α-ketoglutarate:

$$HOOCCH_2CH_2\overset{\overset{O}{\|}}{C}COOH + CH_3CHO \longrightarrow HOOCCH_2CH_2\overset{\overset{OOH}{\|\ |}}{C}C\text{-}CH_3 + CO_2$$

In subsequent studies this metabolite was quantitatively determined after its formation from α-ketoglutarate and acetaldehyde by various rat

tissue homogenate (Bloom et al., 1966). The implication of this finding on the in vivo system has not been explored. However, these studies indicate that acetaldehyde formed from ethanol would be the most likely substrate to enter into a metabolic pathway that was unavailable to acetate or acetyl CoA, thus affecting ultimately the output of CO_2.

From the foregoing discussion, it is evident that catabolic breakdown to CO_2 through TCA cycle may be the major pathway of elimination of the ethyl moiety of EPTC. However, under physiological and chemical stress, the routes that eventually led to the event may differ. Without any specific enzymatic studies, no conclusion can be drawn as to the particular pathway that is affected or the cause of the delayed and erratic pattern of $^{14}CO_2$ elimination.

Radioactivity Recovered in the Urine and Feces

Recovery of the radioactivity in the urine ranged from 8.4 to 16.9% for the lower doses (0.6-3 mg) and from 16.6 to 35.6% for the higher doses (20.6-100.6 mg). Thus the radioactivity recovered in the urine showed an increasing trend with increased doses; whereas the incorporation into $^{14}CO_2$ decreases. This is illustrated in Figure 5 and Figure 6. The results indicate that renal excretion become progressively more important as a means of elimination of large dose of EPTC. It is possible that as the enzyme system

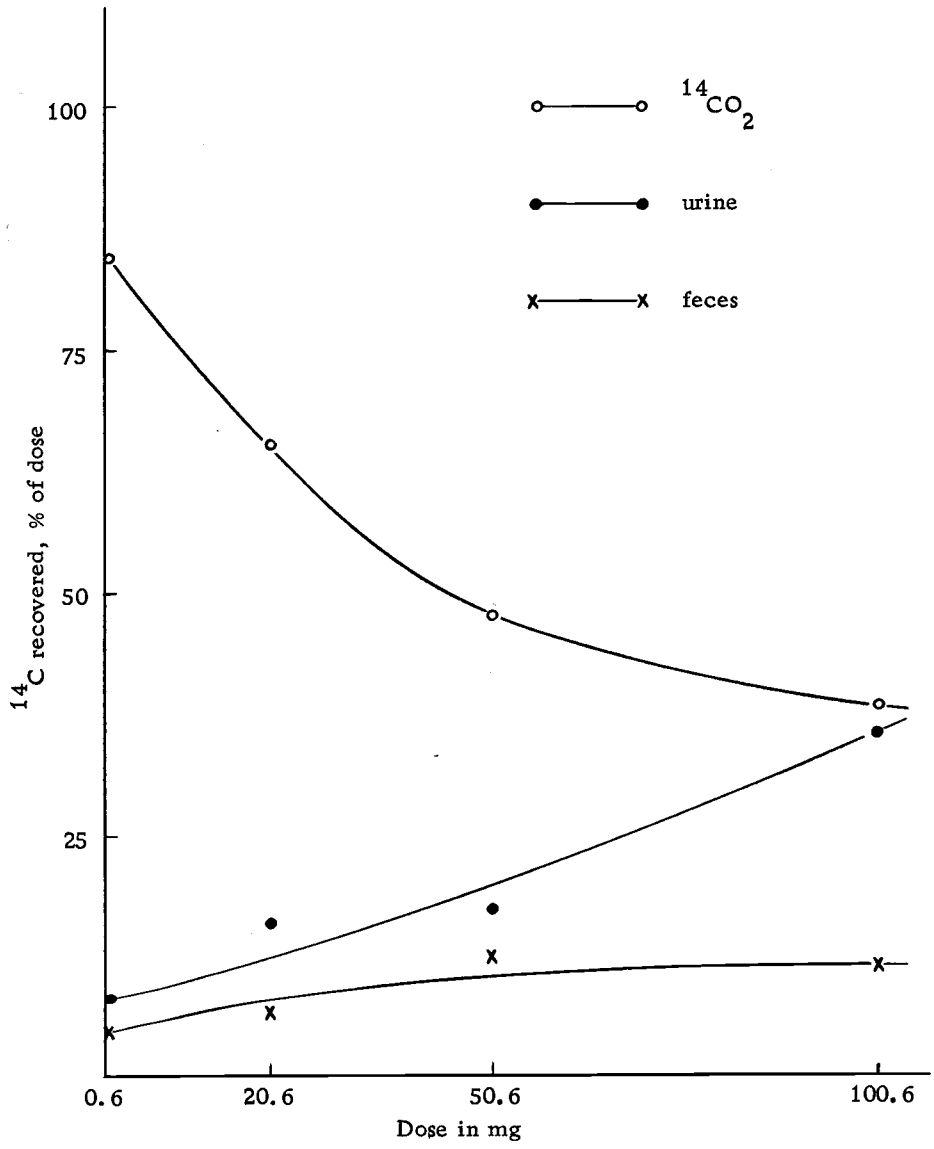


Figure 5. Total ^{14}C recovered in $^{14}CO_2$, urine and feces from female rats treated with varying doses of EPTC-^{14}C.

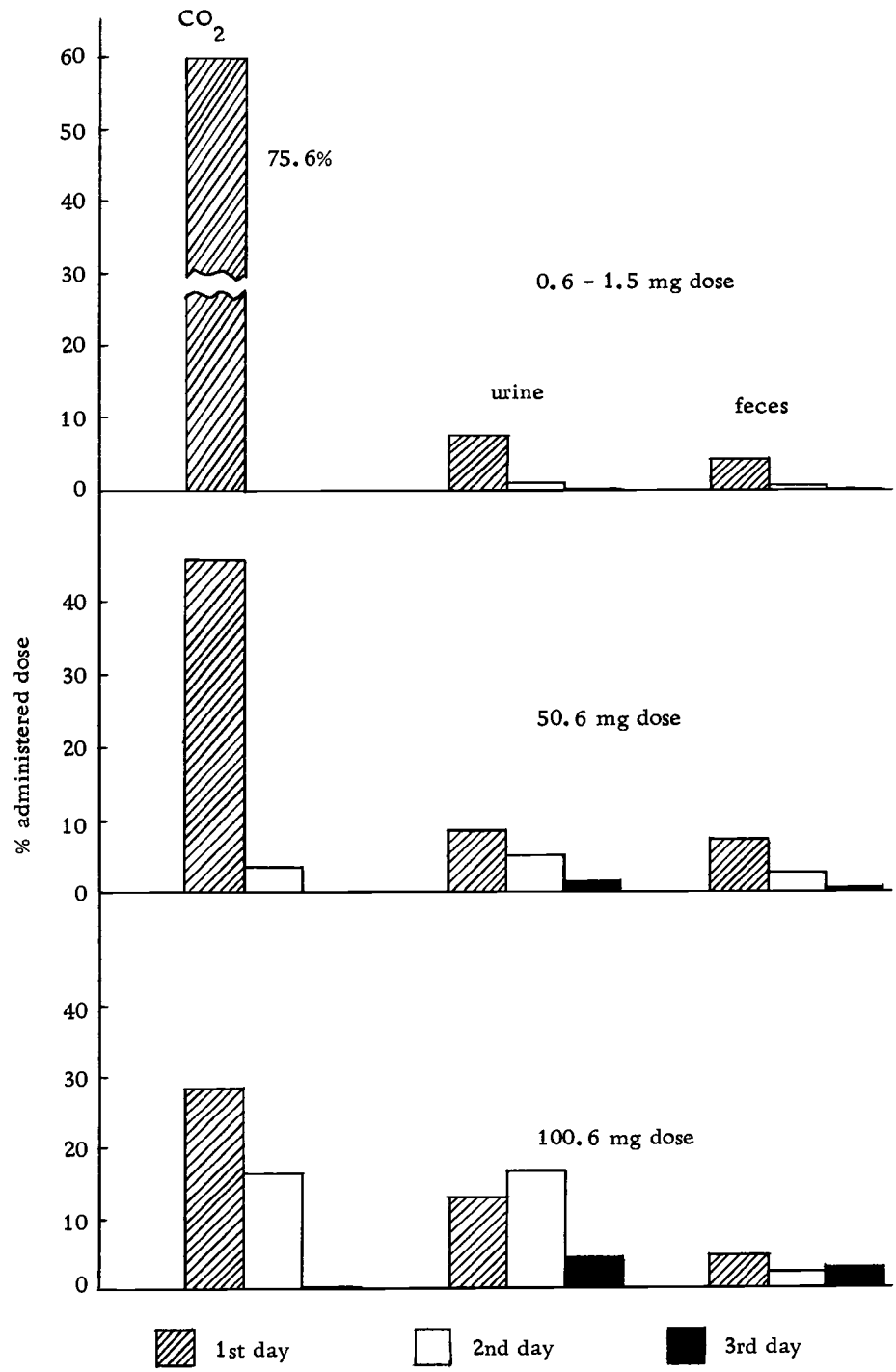


Figure 6. Daily recovery of radioactivity as percentage of given dose.

involved in the catabolic breakdown of EPTC is slowed down at higher doses, there is concomittant increase enzyme activity for the conjugation reaction to take place.

Radioactivity recovered in the feces was generally lower than that of the recovery in the urine. There was also an increasing trend as the doses were increased. However, due to its unavoidable contamination by the urine sample, the value obtained was doubtful. Therefore no analysis of the fecal extract was attempted.

Analysis of the Urinary Metabolites

Steam distillation of the urine sample from rats given 0.6 to 3 mg doses of EPTC-^{14}C revealed that the volatile products in the urine account for 2-4% of the radioactivity of the urine. This indicates that only a small portion of EPTC molecule was excreted unchanged.

The paper chromatographic separation of the radioactive metabolites in the urine sample and their color reactions is summarized in Table 3. The results were the averages from several runs. Of all solvents tested, BAW showed the best separation and distribution of the radioactive peaks. Therefore this solvent was used subsequently for the separation of urine metabolites by paper chromatography.

Radiochromatogram scannings of the paper strips showed that

Table 3. Paper Chromatographic Separation of Radioactive Urinary Metabolites from 24 Hour Urine of Rats Receiving Varying Doses of EPTC-^{14}C. R_f Values, Percent Distribution, and Color Reaction of Metabolites.

	R_f Values				% Distribution								
Metabolite	A	B	C	D	3 mg	53 mg	103 mg	Ninhydrin	Carbohydrate Reagent	Sulfate	Sulfide Sulfoxide Sulfone	Erlich	Naphthoresorcinol Reagent
I	0.0	-	-	-	t	t	t	+	-	-	-	-	-
II	0.03	-	-	-	t	t	t	-	-	-	-	-	-
III	0.11	-	-	-	1.5	1.7	1.2	+	-	+	-	-	+
IV	0.26	0.58	0.55	0.47	25.8	11.9	8.6	-	-	-	-	+	-
V	0.35	0.44	0.61	0.44	3.5	4.4	5.0	-	-	-	-	-	-
VI	0.53	0.51	0.57	0.73	17.4	17.4	17.0	-	-	-	-	++	-
VII	0.66	0.61	0.53	0.46	13.7	24.9	23.7	-	-	-	-	-	-
VIII	0.76	0.79	0.75	0.70	13.7	15.1	23.2	+	-	-	-	-	-
IX	0.88	0.65	0.86	0.83	25.4	26.9	25.2	-	-	-	-	-	-

A. BAW (Butanol:acetic acid:water, 8:2:3, v/v/v).

B. EAW (Ethanol:ammonia:water, 18:1:1,v/v/v).

C. PEW (Propanol:ethyl acetate:water, 6:1:3, v/v/v).

D. Phenol:H_2O (500 gm phenol in 125 ml water, w/v).

t trace

EPTC has undergone extensive biotransformation. There are at least six major and three minor radioactive metabolites in the urine. The presence of the label suggests that the biotransformation product contains either the whole molecule of EPTC or the ethyl moiety of EPTC. The percentage of distribution of three radioactive peaks was affected by the increasing dose. These are metabolites IV, VII and VIII. Metabolite IV showed approximately a threefold decrease when the dose was increased from 3 mg to 100.6 mg, while metabolites VII and VIII showed approximately two fold increase.

Autoradiograms of the paper chromatograms, shown along with the densitometric scanning record in Figure 7, established clearly the results obtained with radiochromatogram scannings. The darkness of the spot in the photographic plate is proportional to the quantity of the radioactive substance present in the area. Since the experimental conditions and the amount of the urine applied to the paper strips were identical, the difference in the intensity of the spot reflects the variation in the percent recovery of the radioactive metabolite due to the variation in doses.

Metabolite VI yielded a characteristic bright yellow color with Ehrlich reagent. When radioactive standard urea was used for co-chromatography with the urine sample, the same R_f value and color reaction with Erlich reagent were obtained in three solvent systems. Therefore metabolite VI was tentatively identified as urea. Since

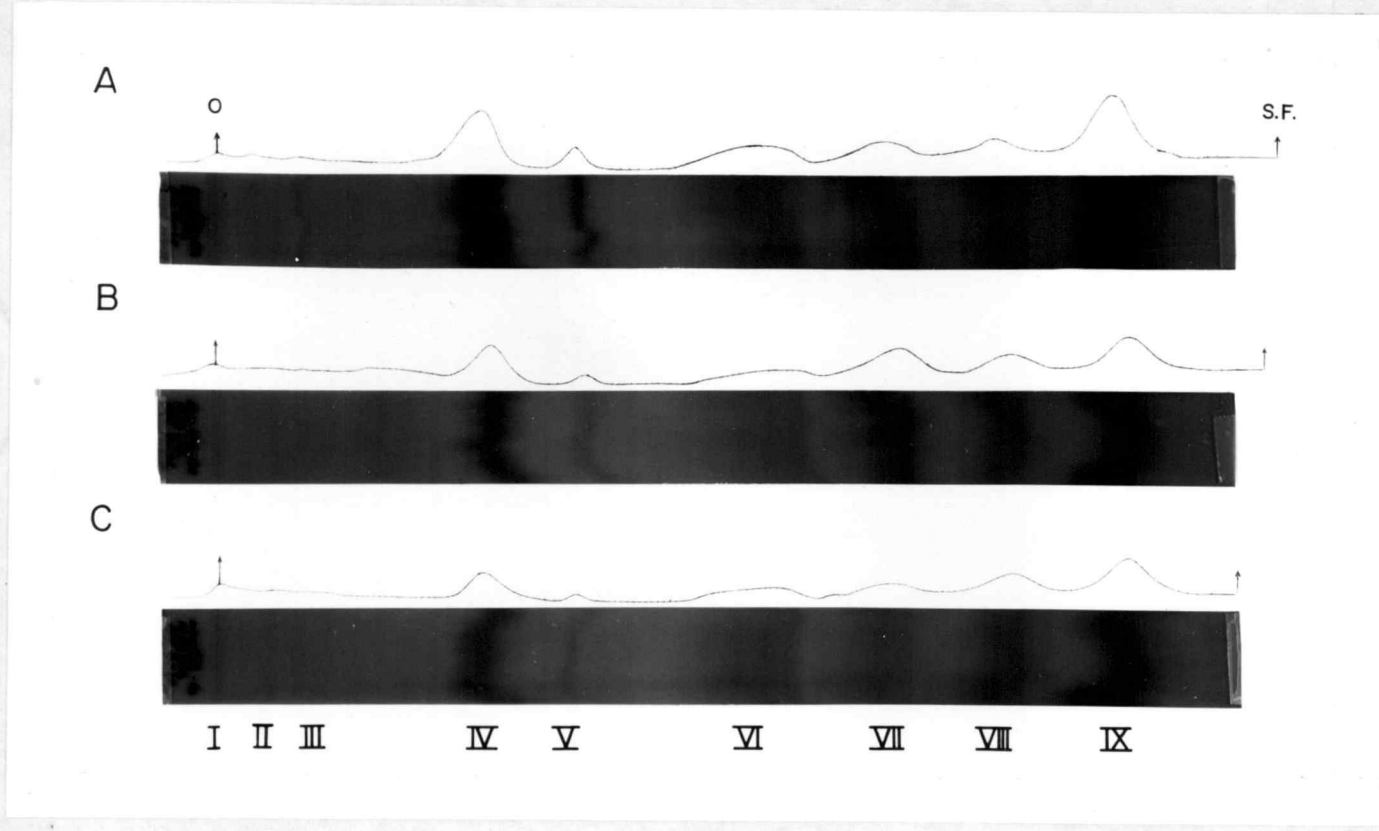


Figure 7. Densitometric scanning record and autoradiograms of radioactive urinary metabolites from rats receiving oral administration of EPTC-^{14}C.

A. 3 mg
B. 53 mg
C. 103 mg

O = origin
S.F. = solvent front

urea has been reported earlier as one of the major metabolite of acetate metabolism in rats (Philleo and Fang, 1968), and of ethyl mercaptan in guinea pig (Snow, 1953; Lowe, 1957), the present finding support the proposed scheme for the ester hydrolysis of thiolcarbamate.

A minor metabolite with R_f value of 0.28 was reported for acetate (Philleo and Fang, 1968; Soo, 1969). This metabolite has been isolated and purified on a Sephadex column. Although still unidentified, the IR study, molecular weight and nitrogen determination seemed to suggest that the compound was a derivative of urea. It was speculated that metabolite IV and the acetate minor metabolite may be identical, since metabolite IV and the minor metabolite from acetate gave very close R_f value in the same solvent system. In addition, a faint positive reaction was obtained with Erlich reagent in the present experiment. However, when the purified acetate metabolite (Soo, 1969) was co-chromatographed with either the EPTC treated urine or the eluted metabolite IV a different result was obtained. Two hundred μg of the acetate metabolite failed to give any color reaction with Erlich reagent. The low activity of the acetate metabolite render further comparison difficult.

Ninhydrin tests showed that introduction of EPTC led to an increase in the output of ninhydrin reactive products. One of the ninhydrin reactive spots (R_f 0.72) drew special attention due to its

absence in the control urine, and the tremendous increase in its color intensity as the doses were increased to 200 mg (Figure 8). The R_f value of this abnormal product was very close to that of metabolite VIII, but closer examination revealed that the two spots are distinct. Autoradiogram established the non-radioactive nature of this metabolite.

Since this ninhydrin reactive metabolite appeared only with the treated urine and increased with the increase doses, it was speculated that either the specific grouping of EPTC, particularly the amine moiety, led to the color reaction with ninhydrin, or the EPTC molecule itself caused the inhibition of a specific enzyme therefore blocking the metabolism of a particular amino acid. Co-chromatography of several known amino acids with urine samples established that none of the common amino acids give as high an R_f value in the BAW solvent system as this abnormal metabolite. The nature of this ninhydrin reactive spot remained unknown.

As a result of this finding, the effect of the amine side chains to bring about the increased ninhydrin reactive abnormal metabolite has been carried out by giving to the rats 100 mg dose each of vernolate and tillam. It was found that both compounds gave rise to a drastic increase in the output of an abnormal compound. However, the R_f value of the spot from the tillam treated urine differed slightly from the one obtained with EPTC and vernolate treated ones.

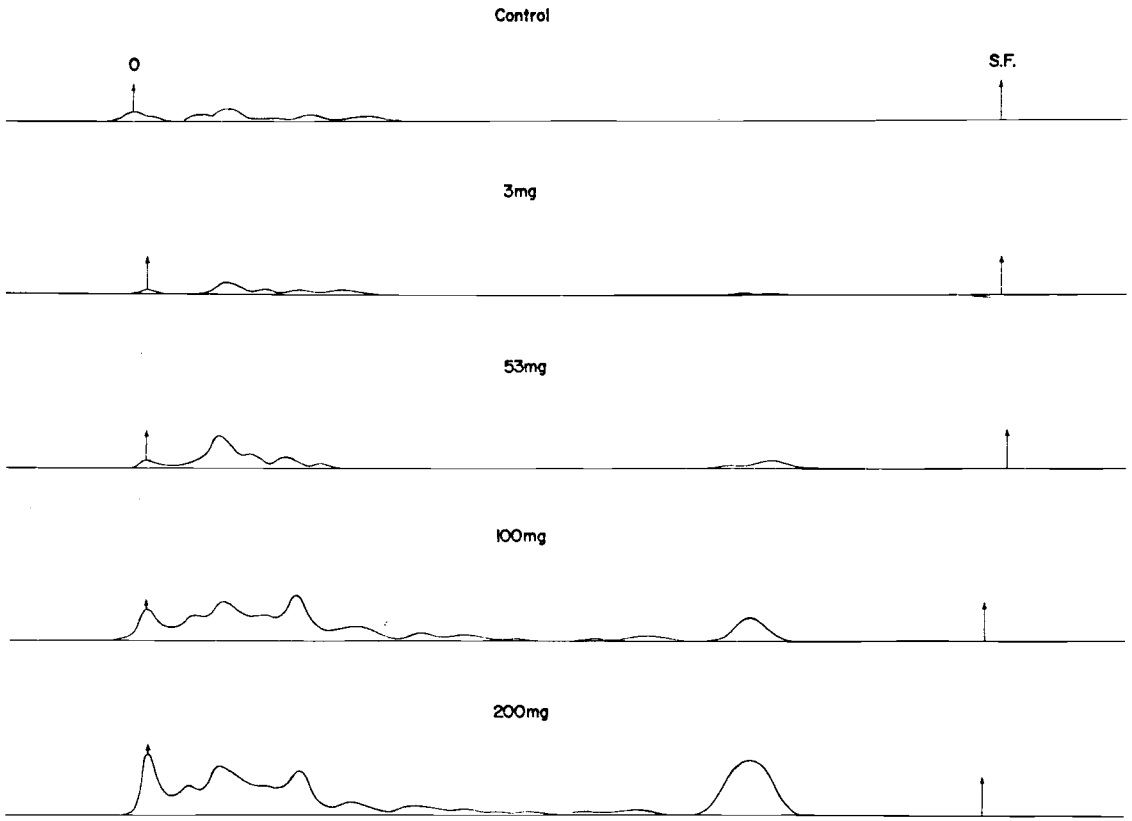


Figure 8. Effect of EPTC on ninhydrin reactive urinary metabolites in rats.

O = origin
S.F. = solvent front

It is therefore believed that the same dipropylamine side chain in EPTC and vernolate might bring about the reaction with ninhydrin. But introduction of 100 mg of dipropylamine directly to the rats produced no effect on the output of amino acids nor the appearance of the abnormal ninhydrin reactive metabolite. Further investigtion of the nature of this abnormal metabolite would probably shed more light on the toxic action of this thiolcarbamate.

Although ethyl methyl sulfone was identified as one of the major metabolites of ethyl mercaptan, none of the major metabolites in the present study responded to the test for sulfone, sulfoxide, and sulfide. The possibility of the methylation of ethyl mercaptan in vivo to ethyl methyl sulfide with subsequent oxidation to sulfone via sulfoxide, however, can not be ruled out.

β-Ethyl-D-glucuronide, which was established by the naphthoresorcinol method and gas liquid chromatography (Jaakonmaki et al., 1968) as a minor metabolite for ethanol in the rabbit, the rat, and man was not observed here. Kamil et al. (1952) noted that although the conjugation process represents only a minor pathway of ethanol metabolism, the amount of this conjugated product, was found to rise with increasing doses. This is in agreement with the present study which showed an increase in radioactivity in the urine as the dose was increased.

Metabolite III, a relatively minor radioactive metabolite, gave

a faint positive reaction with naphthoresorcinol reagent. Positive reactions were also obtained with sulfate and ninhydrin reagent. The nature of this metabolite was also not ascertained.

The failure in this study to obtain color reaction with most of the major metabolites does not rule out the presence of glucuronide, amine, or sulfone as some of the radioactive metabolites in the urine. The negative results may only reflect the inadequacy of the present technique and the low sensitivity of the color method. The functional groupings of the compound could be tied up in linkage formation which would necessitate more elaborate hydrolytic procedures before responding to coloring reagents. Since examination with coloring reagents constitutes only a preliminary test, the results obtained here serve only as a guide for future investigations.

One dimensional thin layer chromatography using either the home made Silica Gel G glass plate or the commercial thin layer ChromAR 500 gave generally a poorer separation of the urinary metabolites as compared with one dimensional paper chromatography. Better resolution of the radioactive metabolites however, was obtained with two dimensional chromatography on both kinds of thin layer plates. Figure 9 depicts the separation of radioactive metabolites by two dimensional chromatographic technique on a Silica Gel G glass plate, using BAW as the first solvent system and isopropanol:formic acid (6N):water (40:2:10, v/v/v) as the second solvent system. Approximately 20 spots, including 7 major ones,

Figure 9. Autoradiogram of two dimensional chromatographic separation of labeled urinary metabolites on Silica Gel G thin layer plate.

were detected with autoradiography; as compared with a total of 9 obtained with one dimensional paper chromatograms. The finding indicates a possible overlapping of some metabolites in one dimensional paper strips.

One of the setbacks encountered in the experiments with hydriodic acid treatment on the thin layer plates was the evolution of iodine gas during the heating process. The iodine gas caused the darkening of the spots thereby obscuring the identification of color. The darkening of the spot by iodine gas can be partially avoided by omitting the use of second glass plate over the thin layer plate. This however, reduces the sensitivity of the color reaction.

Separation of Urinary Metabolites

Extraction of freeze dried urine sample with organic solvents offered no advantage by way of separation of metabolites. It was found that the urine sample used for extraction has to be absolutely dry for the extraction to be effective. If, by any chance, water is reabsorbed by the freeze dried urine during the extraction process, the whole sample becomes very sticky and it is nearly impossible to transfer the sample or effect a good extraction.

Results with descending paper chromatography of the benzene, ethyl acetate, and ethyl alcohol extract further revealed that complete extraction of metabolites with one solvent was not achieved.

Consequently, metabolites that appeared on the extract of one solvent also appeared on the extract of the other. Paper chromatograms showed that three metabolites were extracted by benzene, seven by ethyl acetate, and all metabolites appeared in the ethyl alcohol extract. The effect of water reabsorption on the efficiency of extraction may account for the incomplete recovery of metabolites in various fractions.

Column chromatography of the urine sample likewise produced no encouraging result. All radioactivity was recovered from the cationic column in the water wash. Sixty to 66% of the radioactivity was recovered in the neutral fractions, and 35-43% was recovered in the anionic fractions. Paper chromatography revealed that some metabolites undergo changes on passing through the column. Specifically, metabolite IV was lost almost completely from the neutral and anionic fractions, this loss led probably to the increase of radioactivity of other metabolites and the appearance of some new product. Chromatography of two fractions from two runs yielded no consistent results. Obviously the nature of ion exchange resin caused the irrevocable changes in the structure of some metabolites.

Elution of individual metabolite further supports the results obtained with column chromatography, that is, the lability of some metabolites. Chromatography of the eluate obtained at room temperature on the Silica Gel G thin layer plates using three different solvent

systems showed that two spots were obtained from each of the three eluted metabolites; metabolites VII, VIII, and IX. All spots showed similar color reaction as standard EPTC after treatment with hydriodic acid and ninhydrin spray. They differed only in color intensity. In two solvent systems, one of the two spots from metabolite IX run very close to standard EPTC. It is doubtful that this compound is the free EPTC in the urine sample, since residual EPTC only accounts for 2-4% of the radioactivity in the urine, as indicated by the steam distillation experiment, whereas the radioactivity recovered by the metabolite IX amounts to approximately 26% of the urine activity. It is most probable that all three metabolites contain the intact EPTC molecule which was cleaved off during elution process at the room temperature. The susceptibility to cleavage of metabolites VIII and IX was considerably reduced when elution was carried out in the cold room. However, metabolite VII still suffered cleavage under cold room elution process.

All eluted metabolites are hygroscopic, very soluble in water and sparingly soluble in 95% ethyl alcohol. They are insoluble in most organic solvents such as acetone, isopropanol, or hexane.

Although the isolation of the individual metabolite was not successful, the information was valuable in evaluating the steps that should be taken and avoided in the future investigation.

Analysis of Tissue Accumulation in Various Organs

The accumulation of radioactivity in various internal organs 26 hours after oral administration of EPTC-^{14}C is shown in Figure 10. Of all tissues examined, liver, stomach, lung, and kidney, in that order, showed the highest accumulation of radioactivity. This sequence is to be expected if one considers the route by which the foreign compound is eliminated. The data also showed that increasing the dose increases the accumulation of label in various organs. However, this accumulation may not be detrimental since the elimination proceeds at a rapid rate. The radioactivity in various organs of rats killed 48 hours after dosing has been found to be almost one half of the radioactivity in the organs from rats sacrificed 24 hours after dosing.

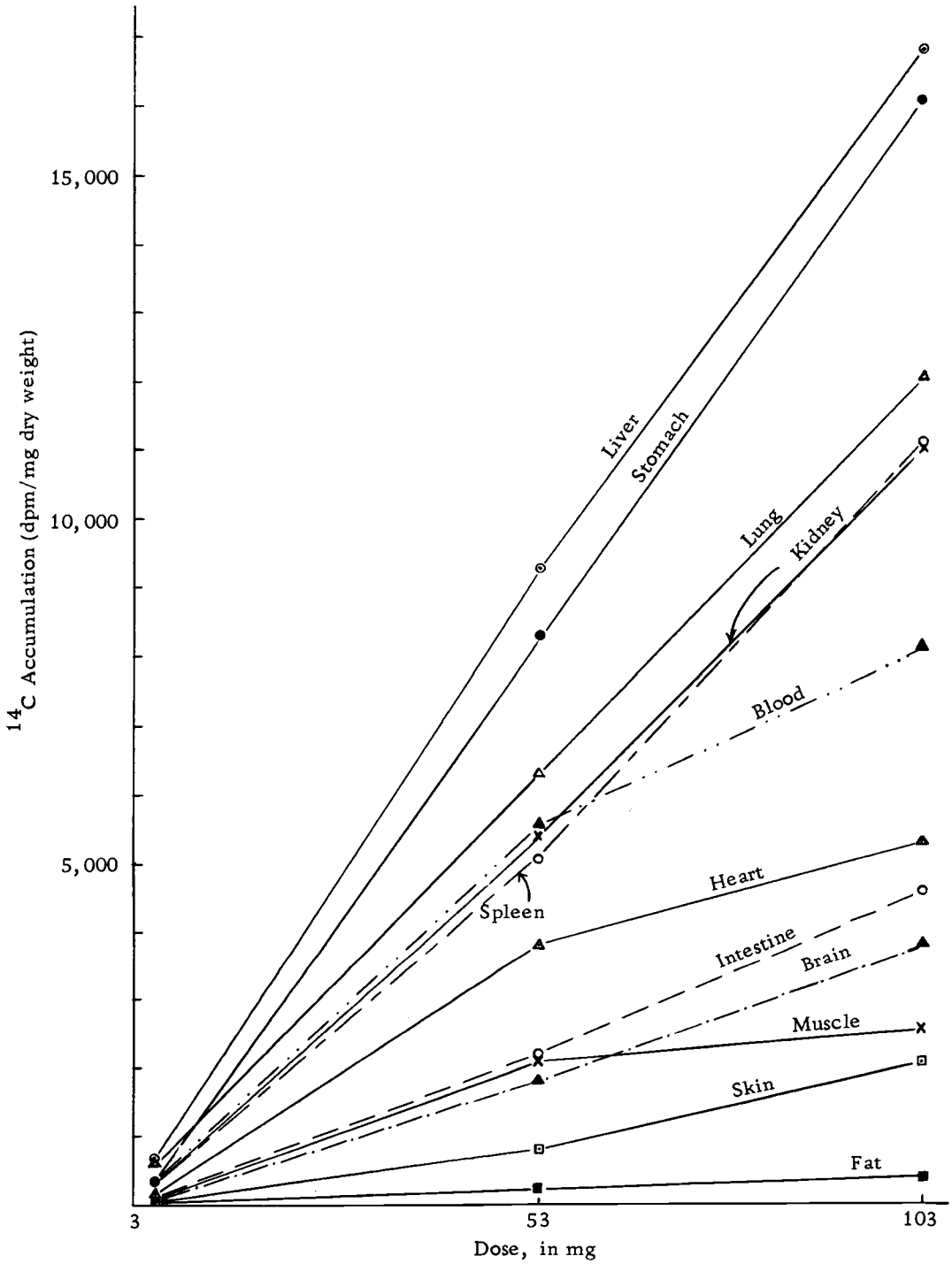


Figure 10. Accumulation of radioactivity in various tissues 26 hours after oral dosing of EPTC-^{14}C.

SUMMARY AND CONCLUSION

EPTC-^{14}C was rapidly metabolized by adult female rats after single oral dosing. The rate of elimination and percentage distribution of ^{14}C in the excreta of rats differed markedly with doses. For 0.6 to 3 mg doses, 64.4 to 84.6% of the ^{14}C was recovered in the respired $^{14}CO_2$ in 15 hours. In contrast, only 38.2% of the ^{14}C was recovered as $^{14}CO_2$ in 35 hours as the doses were increased to 100.6 mg. This decrease in the expired $^{14}CO_2$ with rising doses was accompanied by a corresponding increase in the radioactivity recovered in the urine and feces. Thus 8.4% of ^{14}C was recovered in the urine of rats treated with 0.6 mg of EPTC-^{14}C compared with 35.6% recovery in the urine of rats treated with 100.6 mg of EPTC-^{14}C. These results indicate that while catabolism to $^{14}CO_2$ constitutes the major pathway for the breakdown of EPTC-^{14}C, this pathway was overwhelmed at higher doses to such an extent that renal excretion becomes increasingly important as a mean for the rapid elimination of large doses of EPTC.

Paper chromatographic separation of urine samples and scannings or autoradiography demonstrated that there are at least six major radioactive metabolites and three minor ones. Two dimensional chromatography with a thin layer plate however, revealed a total of twenty radioactive metabolites, of which seven are the major

one. These results indicate that one dimensional chromatography has not effected a complete resolution of the minor metabolites.

Urea was tentatively identified as one of the major metabolites. Metabolite VIII reacts positively to ninhydrin albeit very faintly. Metabolite III, a minor metabolite, reacts positively with ninhydrin, sulfate, and naphthoresorcinol reagents, suggesting that this metabolite may be a glucuronic acid conjugate. Ethyl methyl sulfone, one of the major metabolites reported for ethyl mercaptan, was not detected in the present study.

All metabolites are hygroscopic, very soluble in water and sparingly soluble in 95% ethyl alcohol and insoluble in most organic solvents. The solubility in water indicates the conjugate nature of the product. No extensive characterization of the products was attempted.

Ion exchange column chromatography and elution processes established the lability of some metabolic products. Extraction of metabolites with organic solvents benzene, ethyl acetate, and ethyl alcohol has not been successful.

This study showed that EPTC is rapidly oxidized and eliminated through respiratory route and renal excretion. It is unlikely to constitute a health hazard if incidentally ingested in small doses.

The study is in agreement with findings using tillam-^{14}C, that the breakdown of this thiolcarbamate compound is by way of

initial ester hydrolysis, transthiolation to ethyl alcohol, and catabolism to CO_2. Conjugation mechanism appeared to be the second important pathway for thiolcarbamate elimination.

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