Determination and Disposition of Trifluralin in the Rat. Separation by Sequential High-Pressure Liquid Chromatography and Quantitation by Field Ionization Mass Spectrometry

Henry d'A. Heck,* Robert L. Dyer, Arthur C. Scott, and Michael Anbar

A methodology for analysis of the herbicide, trifluralin, in animal tissues and excreta was developed using stable isotope dilution and field ionization mass spectrometry. Separation of the compound from the tissues was performed by sequential high-pressure gel permeation and reversed-phase liquid chromatography. The technique was applied to rat tissues following intraperitoneal injection or chronic oral administration of the herbicide. A two-compartment pharmacokinetic model is used to describe the uptake and disappearance of trifluralin in liver and fat following injection.

Substituted dinitroaniline herbicides are an economically important class of agricultural compounds used to prevent the growth of grasses and weeds in cultivated crops. Trifluralin (Treflan) (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine (TFN)), the most heavily consumed herbicide of this class, is extensively applied to soybean, alfalfa, and cotton fields in southern and midwestern areas of the United States as well as in other countries. The consumption of TFN in 1975 exceeded 20×10^6 lb (F. Chan, Chemical Information Services, Stanford Research Institute, personal communication).

The possibility that TFN might enter the food chain, either through consumption by foraging livestock or by direct application to edible crops, has prompted studies of the distribution and metabolism of TFN in animals and plants (Emmerson and Anderson, 1966; Golab et al., 1969; Plimmer and Klingebiel, 1974). Methods that have been used to assay for TFN in tissues or in soils include thin-layer chromatography (Golab et al., 1969), gas–liquid chromatography (Tepe and Scroggs, 1967; Hambleton, 1971; Aue et al., 1973; Smith, 1974; Payne et al., 1974; Hall and Mallen, 1976), gas–liquid chromatography/mass spectrometry (Downer et al., 1976), and spark source mass spectrometry (Tong et al., 1972).

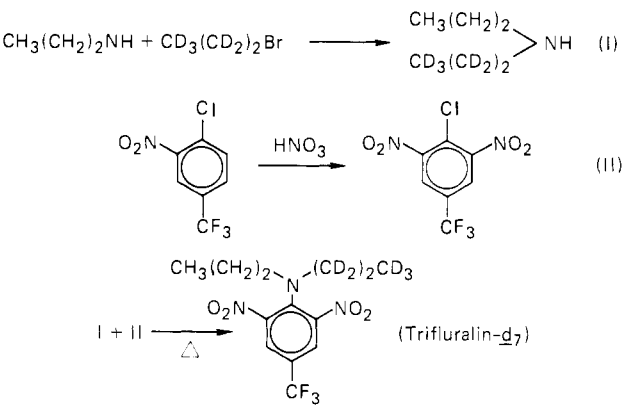
Isotope dilution analysis using nonfragmenting mass spectrometry offers several advantages over other techniques for determining trace quantities of organic compounds in biological materials (Anbar and Aberth 1974). This paper describes a technique for the determination of TFN in rat tissues and excreta using isotope dilution analysis in conjunction with sequential high-pressure liquid chromatography (HPLC). The methodology was developed to study the distribution and persistence of TFN in rats following injection or chronic oral administration of the herbicide. Data from single injections in this exploratory study were analyzed by computer in terms of a two-compartment pharmacokinetic model, and half-lives were estimated for the disappearance of TFN from rat liver and fat.

MATERIALS AND METHODS

Chemicals. Unlabeled TFN was obtained from Eli Lilly and Co., Indianapolis, Ind. Heptadeuterated TFN was prepared by the reactions shown in Scheme I.

The synthesis of *n*-propyl-*n*-heptadeuteriopropylamine (I) was achieved by refluxing *n*-propylamine (Eastman) with *n*-heptadeuteriopropyl bromide (Merck) in the presence of 4 M sodium hydroxide. Distillation of the product at 110 °C yielded an azeotropic mixture that, after drying with NaOH pellets, was redistilled at 108–110 °C to obtain the di-*n*-propylamine-d_7.

Scheme I



3,5-Dinitro-4-chlorobenzotrifluoride (II) was prepared by dropwise addition of a sulfuric/nitric acid solution to a mixture of 3-nitro-4-chlorobenzotrifluoride in oleum (Yagupol'skii and Mospau, 1955). The resulting mixture was heated at 110 °C for 3.5 h, then it was cooled and poured over ice, and the precipitate was collected, washed, and dried under vacuum. Recrystallization from ethanol gave an 85% yield of the product (mp 58 °C).

TFN-d_7 was synthesized by heating a mixture of 3 mL of I and 2.4 g of II at 100 °C for 2 h (Soper, 1966). The mixture was dried with anhydrous ether, the amine hydrochloride removed by filtration, and the filtrate concentrated to give the crude product. Recrystallization from hexane yielded 1.3 g (43%) of the final product, the identity of which was confirmed by infrared and proton magnetic resonance spectroscopy.

The field ionization mass spectrum of the synthetic TFN-d_7 is shown in Figure 1. The predominant isotopic variant occurs as expected at a mass/charge (m/e) ratio of 342, corresponding to TFN-d_7. A minor peak is also present at m/e 335, corresponding to the mass of unlabeled TFN. High-pressure liquid chromatography using an octadecylsilane bonded stationary phase (see below) failed to separate the contaminant at m/e 335. This result indicates that the minor peak at m/e 335 is due to unlabeled TFN.

The source of unlabeled TFN in the TFN-d_7 was traced to the *n*-propylamine, which was shown by mass spectrometry to contain a similarly small amount of di-*n*-

Mass Spectrometry Research Center, Stanford Research Institute, Menlo Park, California 94025.

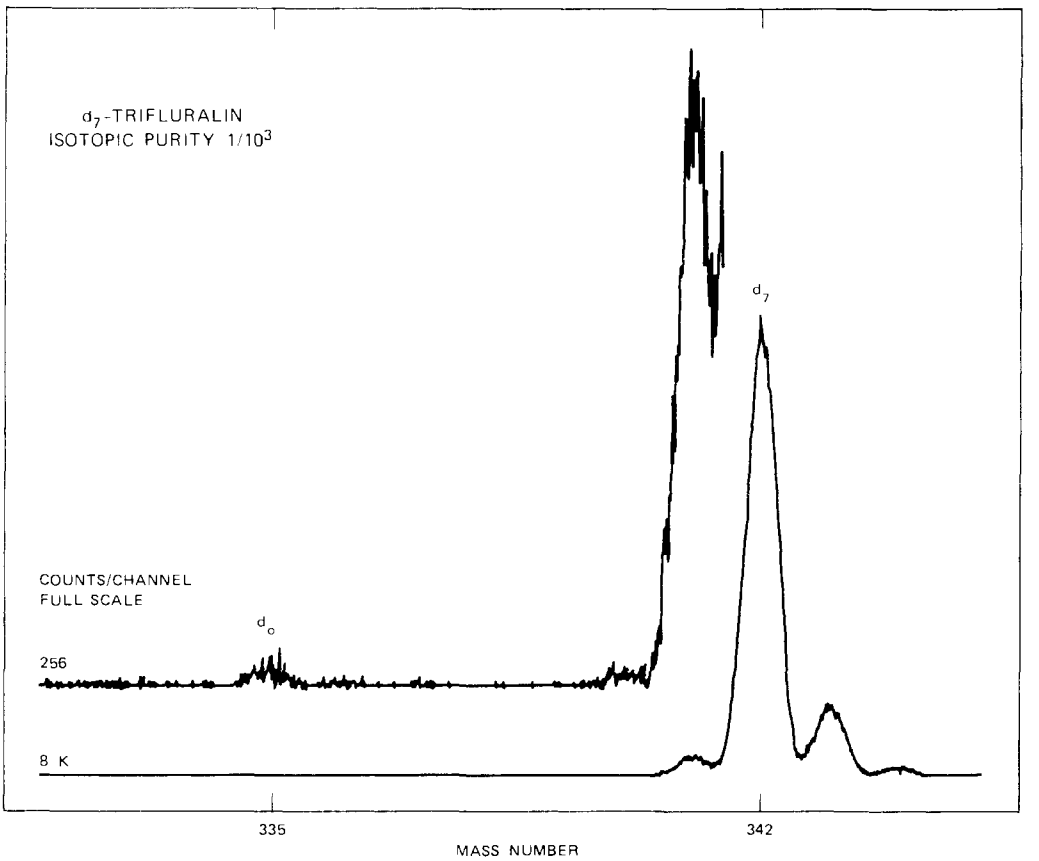


Figure 1. Field ionization mass spectrum of trifluralin-d_7.

propylamine. A resynthesis of TFN-d_7 in an isotopically pure form was not attempted for this study. Instead, we used the material as prepared for animal injections and feedings, and unlabeled TFN was the carrier in the isotope dilution analysis.

Organic solvents were obtained from Burdick and Jackson Laboratories, Muskegon, Mich. Water was distilled from alkaline permanganate before use.

Instrumentation. Cleanup of organic extracts was performed using a high-speed, multi-column gel permeation chromatograph constructed in this laboratory. The apparatus consists of a Haskel pneumatic amplifier pump, a flow-through 0–5000 psi Helicoid gauge, a Rheodyne variable volume injector, and three Waters μ-Styragel columns (30 cm × 7 mm i.d.), 100-Å pore, connected in series. A dual wavelength ultraviolet absorbance detector (Spectra-Physics) connected to a dual channel strip chart recorder monitored the column effluent simultaneously at 254 and 280 nm. Following the detector, a Rheodyne rotary valve was used either to manually collect an individual peak or to direct the flow of solvent to waste.

Purification of TFN from the appropriate gel permeation chromatography (GPC) fraction was performed by high-pressure liquid–liquid chromatography (LLC) using a Waters Model 6000A HPLC apparatus equipped with a μ-Bondapak C_{18} column and a Model 440 absorbance detector.

Mass spectrometric measurements were carried out using a single focusing mass spectrometer constructed in this laboratory equipped with a 90° sector magnet. Molecular ions were produced with a multipoint field ionization source operating at an ionizing potential of 1.5 kV (Anbar and Aberth, 1974). To record the ratio of labeled to unlabeled TFN, the molecular ion region was scanned repetitively by varying the accelerating voltage, and the electron multiplier output was integrated by a multichannel analyzer. The analyzer sweep controls the scan via a programmable power supply. Samples are introduced into the spectrometer with a solid sample inlet probe (McReynolds et al., 1975).

Animals. Thirty male rats (Sprague-Dawley) of age 60 days were used in the study. The average weight of the animals was 338 ± 18 (SD) g. The rats were allowed to acclimate in cages for 4 days before the experiments were begun. Administration of TFN-d_7 was either by intraperitoneal (ip) injection or by oral gavage.

Intraperitoneal Administration. Five groups of three rats each received single injections of TFN-d_7 dissolved in sesame oil ([TFN-d_7] = 500 μg/mL). The injections were placed in the lower peritoneum at a dose of 500 μg/kg. Three control rats received similar injections of sesame oil at a level of 1 mL/kg. All rats were maintained in individual metabolism cages.

Groups of three test rats were sacrificed by asphyxiation in a CO_2 atmosphere at 4, 8, 24, 48, and 72 h post-injection. The control rats were sacrificed at 4, 24, and 72 h post-injection. Urine and feces were collected from rats sacrificed at 24, 48, and 72 h post-injection during the 24-h period preceding sacrifice. At the time of sacrifice, fat from the upper abdomen, liver, and blood from each rat were obtained. The blood was collected in heparinized tubes, centrifuged, and the plasma was stored in dry ice. All tissues and excreta were weighed (or the volumes were measured) and then were frozen in dry ice.

Oral Administration. Three groups of three rats each received TFN-d_7 daily by oral gavage for 21 consecutive days. The TFN-d_7 was dissolved in a vehicle composed of 9 g of NaCl, 4 mL of polysorbate, 5 g of carboxymethylcellulose, and 9 mL of benzyl alcohol at three TFN-d_7 concentrations: 20, 100, and 500 μg/mL. The rats were weighed and the administered volumes were adjusted

daily so that the three groups received TFN-d_7 at dosages of 20, 100, and 500 μg/kg, respectively. A fourth group of three rats served as the control, and received only the vehicle at a level of 1 mL/kg daily. The rats were housed 3 per cage and received food and water ad libitum.

Twenty-four hour urine and feces samples were collected from all rats on days 7, 14, and 21. The rats were placed individually in metabolism cages for 24 h and were returned to gang cages after the samples were obtained.

All rats were sacrificed on the 22nd day after the treatment began. Fat, liver, and blood were collected at the time of sacrifice and were treated as above.

Extraction. One gram each of fat, liver, or feces, or 1 mL of plasma or urine was used per sample. One milliliter of 1.5 M potassium phosphate buffer (pH 6.8) was added to plasma or urine before extraction. To each sample was added 80 μg of unlabeled TFN. Tissue and liquid samples were homogenized for 30 s in 8 mL of hexane/ether (9:1) using a Tekmar Tissumizer. The homogenates were centrifuged for 5 min at approximately 1500*g*, and the organic layer was filtered through a Millipore filter. Some plasma samples required the addition of approximately 0.5 mL of isoamyl alcohol to break emulsions.

Feces were extracted after initially suspending the feces in 8 mL of distilled water and 1 mL of phosphate buffer in an Erlenmeyer flask, and allowing the suspension to stand for 30 min. Eight milliliters of hexane/ether (9:1) were subsequently added, and the mixture was vigorously shaken by hand for 30 s. After centrifugation, the organic layer was removed and filtered.

Cleanup and Purification. The TFN extracts were concentrated under a gentle N_2 stream, then were redissolved in tetrahydrofuran (THF) and brought to a final volume of 2 mL. A 0.5-mL aliquot of this solution was injected into the GPC apparatus. The operating pressure was 1000 psi which yielded a flow rate of 1.3 mL/min. TFN peaks were collected manually into Teflon-capped culture tubes.

Peak areas were measured with the aid of a planimeter and were used to calculate the extraction efficiencies from the various biological samples. The calculated extraction efficiencies were essentially independent of the nature of the sample. The average efficiency for all sample types was 70 ± 12 (SD) %.

Solvent was evaporated from the TFN fraction with a stream of dry N_2, and the residue was redissolved in 0.5 mL of H_2O/CH_3OH (3:7, v/v). Eluates from some of the fat samples would not dissolve in this solvent. These samples were suspended in 100% methanol, shaken until all the TFN dissolved, and then were centrifuged to remove fat globules. The supernatant solutions were concentrated under N_2, and the residues were dissolved in H_2O/CH_3OH.

Isocratic reversed-phase chromatography was carried out in 30% H_2O/CH_3OH at a flow rate of 2 mL/min. The TFN peak was collected in a clean culture tube in approximately 2 mL of solvent. Sufficient solid NaCl was added to saturate the solution and this was followed by the addition of 2 mL of cyclopentane. The tubes were capped and shaken by hand for 15 s. TFN was transferred essentially quantitatively to the organic layer, which was removed and stored in a small culture tube until analyzed by mass spectrometry.

Typical chromatograms for the cleanup and purification of TFN by high-pressure gel permeation and reversed-phase partition chromatographies are shown in Figure 2. The distinct TFN chromatographic peaks are due to the addition of unlabeled carrier, which was present in large excess over the residual TFN-d_7 in the sample.

Isotope Ratio Measurements. The purified solution of TFN in cyclopentane was concentrated to a few drops with a gentle flow of dry N_2, and was then placed dropwise on the outside of a sealed glass capillary. Residual solvent was allowed to evaporate, and the loaded capillary was inserted into a cooled, quartz solid sample probe. The latter was introduced into the mass spectrometer through a vacuum lock. Samples were allowed to warm gradually, and recording was begun when the average count rate began to rise above the background. When the sample was exhausted, the spectrum was displayed and the total counts in each mass region were tabulated. Counts were accumulated over a temperature range of 0–10 °C. A complete run required approximately 10 min.

Field ionization mass spectra obtained from chromatographically purified extracts of control rat tissues and of rats injected with TFN-d_7 are shown in Figure 3. A logarithmic scale was used to display the peak intensities. The actual interpeak resolution is equivalent to that shown in Figure 1. The low, mass-unresolved background in the three control samples is caused by ion scattering in the mass spectrometer. There are no detectable chemical impurities in the mass region of interest, which demonstrates the effectiveness of the sequential HPLC technique. TFN-d_7 was not found as such in the plasma of most of the injected rats, but it was detected in liver and fat (Figure 3). Similar spectra to those shown in Figure 3 were obtained from rats that received TFN-d_7 daily for 21 days by oral gavage.

RESULTS

Analytical Sensitivity. A log–log calibration plot comparing measured TFN-d_7/TFN isotopic ratios in solutions of cyclopentane vs. expected ratios was linear over at least three orders of magnitude (slope = 0.99 ± 0.02 (SD); r = 0.999). The lowest concentration of TFN-d_7 measured in this plot was 0.5 ng/mL. Concentrations that appear to be less than 0.5 ppb were regarded as not detectable, and are indicated by ND in the tabulated data (see below).

Intraperitoneal Administration. Following injection of 500 μg/kg of TFN-d_7, the compound was detected at high concentrations in fat and at lower concentrations in liver. TFN-d_7 was not present at detectable levels in the plasma samples of most of the animals. Trace amounts were found in the 24-h urine collections of rats sacrificed 1 day postinjection. The results are summarized in Table I.

All measurements given in Table I were run in duplicate, and the average values are reported. There was good agreement between duplicate runs in the liver series, the average deviation from the mean being approximately 7%. Duplicate fat samples containing higher levels of TFN-d_7 exhibited an average deviation from the mean of only 2%. Larger average deviations, 30 and 44%, respectively, were found, however, in urine and feces samples, where the concentrations of TFN-d_7 were practically at the detection limit. One rat sacrificed at 8 h had no measurable TFN-d_7 in either liver or fat, probably because of an accidental failure to inject.

Oral Administration. Following chronic oral feedings of TFN-d_7 at dose levels of 20, 100, and 500 μg/kg daily for 21 days, the compound was detected at low concentrations in fat in essentially all of the animals and in feces only at the highest dosage. The data are compiled in Table II. Many of the urine samples at the 500 μg/kg dosage level appeared to contain trace amounts of TFN-d_7. The compound was not found in liver, and its apparent

Table I. Concentrations of TFN-d_7 in Rat Tissues and Excreta and Feces Weights or Urine Volumes Following Ip Injection at 500 μg/kg[a]

Time of sacrifice, h post-injection	Rat no.	Trifluralin-d_7 concn and sample wt or vol				
		Liver, ng/g	Fat, ng/g	Plasma, ng/mL	Urine, ng/mL; mL	Feces, ng/g; g
	1	42.7	597	2.3		
4	2	47.6	263	ND		
	3	64.3	392	ND		
	4	ND[b]	ND[b]	ND		
8	5	23.3	256	2.9		
	6	8.1	665	ND		
	7	27.3	584	ND	1.7; 11.0	2.7; 9.8
24	8	11.5	1727	ND	4.1; 8.5	ND; 10.0
	9	9.7	2365	ND	0.6; 7.5	ND; 9.3
	10	1.2	857	ND	0.5; 17.5	ND; 13.9
48	11	7.1	625	ND	ND; 12.5	ND; 11.4
	12	7.8	1292	ND	0.7; 16.0	ND; 11.4
	13	1.1	1913	ND	ND; 13.3	1.6; 17.1
72	14	2.5	84	ND	1.0; 9.8	0.5; 10.5
	15	ND	723	ND	ND; c	5.5; 10.1

[a] Each concentration represents the mean of two determinations. Average deviations from the mean were 7% in liver, 2% in fat, 30% in urine, and 44% in feces. Concentrations below the detection limit of 0.5 ppb are signified by ND (not detectable). [b] Rat apparently not injected with TFN-d_7. [c] 10 mL of H_2O added to concentrated urine to collect sample.

Table II. Concentrations of TFN-d_7 in Rat Tissues and Excreta and Feces Weights or Urine Volumes following Daily Oral Administration for 21 Days[a]

TFN-d_7 dosage, μg kg^{-1} day^{-1}	Rat no.	Trifluralin-d_7 concn and sample wt or vol								
		Liver, ng/g	Fat, ng/g	Plasma, ng/mL	Urine ng/mL; mL) on day			Feces (ng/g; g) on day		
					7	14	21	7	14	21
	1	ND	1.6	ND	5.1; 6.5	ND; 8.5	ND; 8.0	ND; 8.4	ND; 8.5	ND; 6.4
20	2	ND	ND	0.6	ND; 10.5	ND; 7.5	ND; 8.0	ND; 7.7	ND; 9.6	ND; 8.4
	3	ND	4.9	2.7	ND; 10.5	ND; 10.0	ND; 11.6	ND; 8.0	ND; 5.0	ND; 5.7
	4	ND	6.5	ND	ND; 12.5	ND; 12.0	ND; 15.0	ND; 11.2	ND; 10.0	4.7; 8.7
100	5	ND	2.5	ND	ND; 12.5	ND; 9.0	ND; 9.5	ND; 7.7	ND; 8.4	ND; 7.5
	6	ND	18.5	ND	ND; 15.0	ND; 8.0	0.6; 11.3	0.5; 12.1	ND; 10.4	ND; 6.1
	7	ND	16.3	ND	ND; 12.0	1.8; 13.5	2.0; 10.5	6.2; 9.1	192; 12.6	29.5; 4.9
500	8	ND	3.5	1.1	7.9; 7.0	ND; 6.5	1.4; 10.0	2.3; 9.5	7.5; 9.5	61.3; 8.4
	9	ND	9.2	ND	0.5; 11.0	1.2; 15.0	ND; 9.0	75.6; 5.0	2.6; 5.0	3.6; 5.2

[a] Samples that appeared to contain TFN-d_7 were repeated. The average value in such cases is listed. Concentrations below the detection limit of 0.5 ppb are signified by ND (not detectable).

presence in plasma at low concentration in a few samples may be erroneous. Duplicate runs were performed only on those samples that appeared to contain TFN-d_7.

DISCUSSION

Cleanup of Animal Extracts by High-Pressure GPC. The cleanup of organic extracts prior to analysis is an essential step in the determination of pesticide residues in many animal tissues, owing to the relatively large amounts of lipids that may be coextracted. Conventional methods, described by Burchfield and Johnson (1965), involve partitioning between polar and nonpolar organic solvents and/or sorption on columns of charcoal, Florisil, or other materials. Batchwise or continuous elution yields the pesticide in a sufficiently pure state to permit subsequent analysis.

When applied to large numbers of samples, these methods are extremely tedious. As an alternative, several workers have investigated the feasibility of using GPC for sample cleanup. The ability of conventional GPC to separate pesticides from lipids on columns of Bio-Beads was demonstrated by Stalling et al. (1972). Other investigators have used Sephadex LH-20, but the separations obtained with this gel seem to have been inferior to those achieved with Bio-Beads SX-2 (Ruzicka et al., 1968; Pflugmacher and Ebing, 1974).

High-pressure GPC is a more efficient chromatographic technique than conventional GPC. In the apparatus constructed for this study, the theoretical plate count was 3000–5000 for compounds having molecular weights between 100 and 400. Moreover, sample throughput was rapid, and the pesticide was eluted in a small volume, thus simplifying sample collection and handling.

The technique described for the cleanup of trifluralin has also been applied to parathion. Results were equally satisfactory and suggest that high-pressure GPC may be a preferred cleanup method for many types of pesticide residue analysis.

Intraperitoneal Administration. *Pharmacokinetics.* The uptake and disappearance of TFN-d_7 from liver and fat following a single ip injection were analyzed in terms of a two-compartment model. It was assumed that following injection, the liver was rapidly exposed to the bulk of the injected herbicide. Since the blood vessels that drain the peritoneal cavity empty into the hepatic portal vein, this assumption is reasonable. The possibility exists, however, that a significant portion of the injected material could have remained localized at the site of injection (Emmerson and Anderson, 1966). To minimize the influence of such material on the apparent kinetics of trifluralin in adipose tissue, fat samples were taken from sites remote from the injection region.

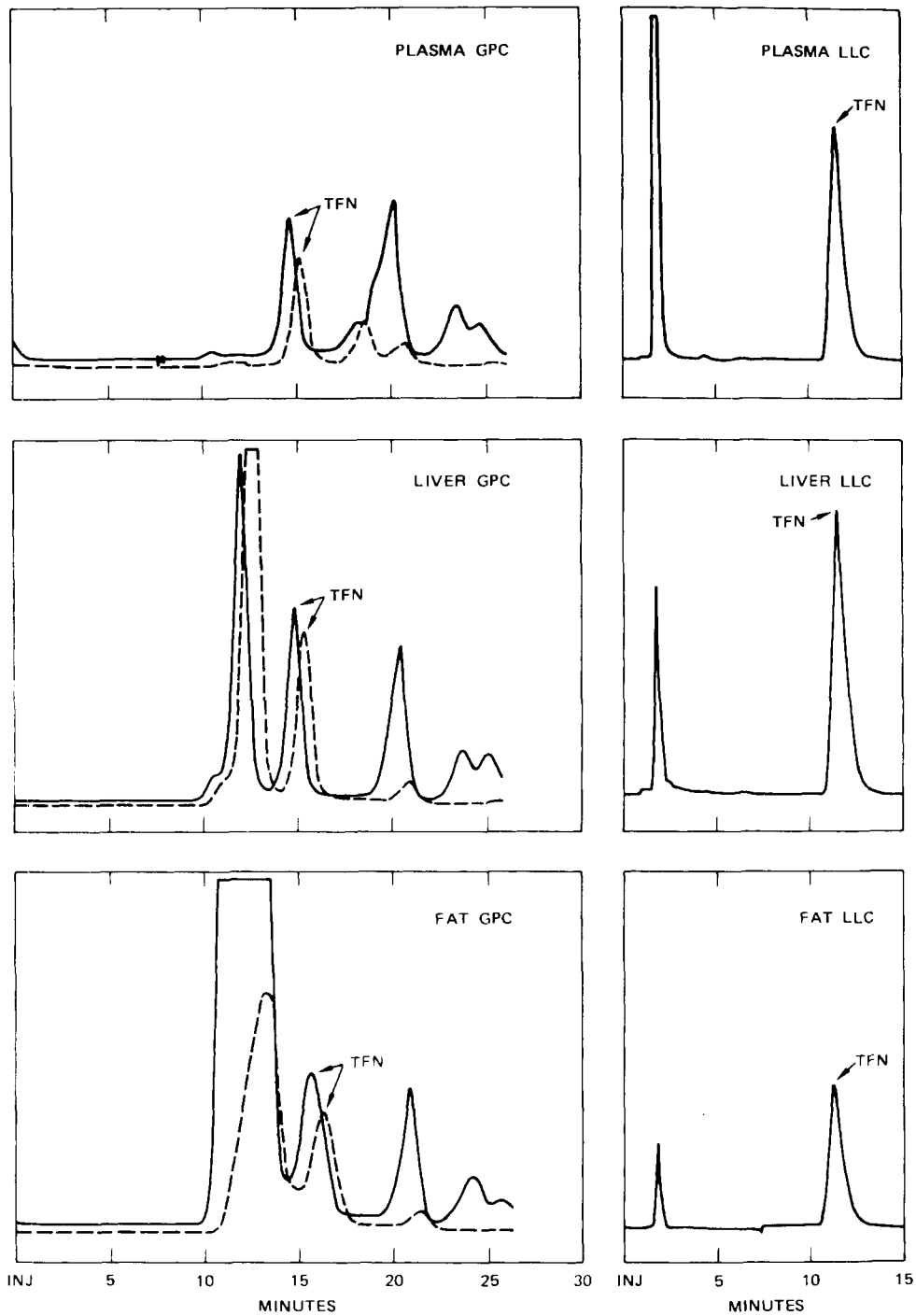


Figure 2. Representative chromatograms for cleanup by high-pressure gel permeation chromatography (GPC) and purification by reversed-phase liquid–liquid chromatography (LLC). GPC conditions: injection volume, 0.5 mL; solvent, THF; flow rate, 1.3 mL/min; absorbance maximum, 0.32; (—) absorbance at 254 nm; (---) absorbance at 280 nm. GPC peaks at 254 and 280 nm do not coincide owing to recorder pen displacement. LLC conditions: injection volume, 0.5 mL; solvent, 3:7 H_2O/CH_3OH; flow rate, 2 mL/min; absorbance maximum, 0.2; (—) absorbance at 254 nm.

Injected material that enters the circulatory system is distributed rapidly among the well-perfused tissues and organs of the animal, including liver, lung, heart, spleen, and, frequently, brain. These tissues, together with plasma, are conventionally assumed to constitute a single compartment, the so-called "central" compartment, within which the foreign compound is equilibrated rapidly according to its affinity for the different tissues. (The kinetic similarities of the different tissues in the compartment are presumably caused by similarities in blood flow and in partition coefficients between blood and tissue (Riegelman et al., 1968).) Since TFN-d_7 was not detected in plasma at times equal to or greater than 4 h, it must be assumed that the herbicide is strongly tissue bound.

During and after the initial uptake of TFN-d_7 by the well-perfused organs, the compound will be more slowly adsorbed by the less vascularized tissues, such as muscle and fat. The kinetic behavior of muscle and fat with respect to lipophilic drugs is markedly different (Brodie, 1964; Chen and Andrade, 1976), and it is therefore incorrect to consider these tissues together as members of the same "peripheral" compartment. The lipophilicity of TFN and its high concentration in fat (Table I) indicate that adipose tissue alone may be regarded as constituting that compartment for our purposes.

These considerations suggest a model such as depicted

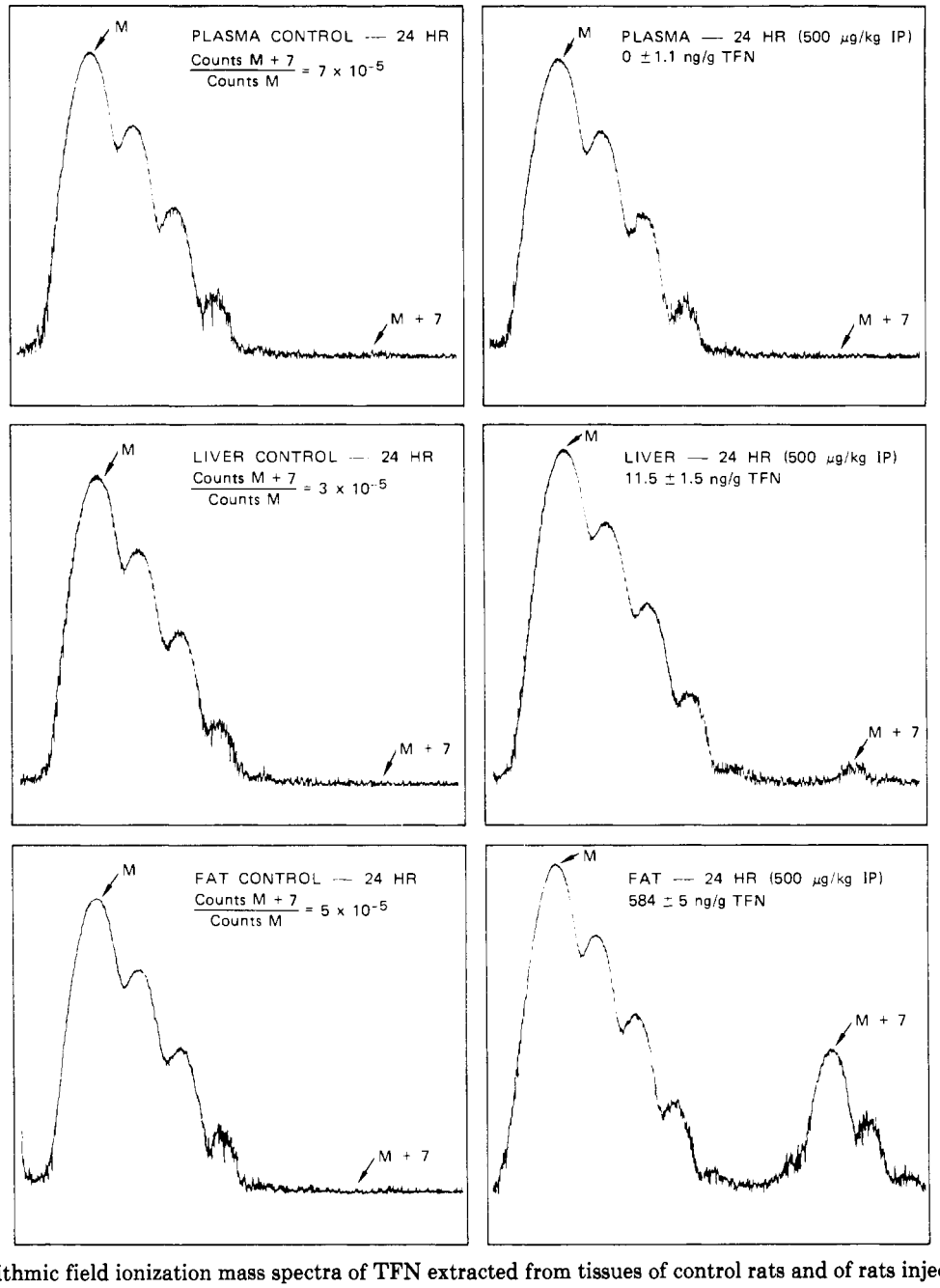
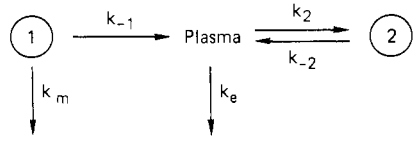


Figure 3. Logarithmic field ionization mass spectra of TFN extracted from tissues of control rats and of rats injected with TFN-d_7 (500 μg/kg).

Scheme II



in Scheme II. In this model, compartment 1 represents the well-perfused tissues while compartment 2 stands for adipose tissue. Uptake of TFN-d_7 by compartment 1 is regarded as being complete by the time of the first analysis (4 h). The tissues of compartment 1 slowly release TFN-d_7 to the plasma (k_{-1}) or metabolize it to various derivatives (k_m) (Emmerson and Anderson, 1966; Plimmer and Klingebiel, 1974). TFN-d_7 in plasma may be either excreted (k_e) or may be absorbed by compartment 2 (k_2). The rate constant for release of TFN-d_7 from compartment 2 is signified by k_{-2}.

Assuming a steady state in the plasma concentration, the integrated expressions for the concentrations of TFN-d_7 in compartments 1 and 2, respectively, become:

$$C_1 = C_1^0 \exp[-(k_{-1} + k_m)t] \quad (1)$$

$$C_2 = \frac{k_{-1}k_2C_1^0}{(k_2 + k_e)(k_{-2} + k_m) - k_{-2}k_e} (\exp\{-[k_{-2}k_e/(k_2 + k_e)]t\} - \exp[-(k_{-1} + k_m)t]) \quad (2)$$

where C_1^0 is the initial concentration of TFN-d_7 in compartment 1 following injection, and the first-order or pseudo-first-order rate constants in the two equations are as defined above.

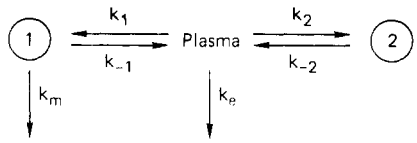
These equations predict that a single exponential will suffice to describe the disappearance of TFN-d_7 from compartment 1, but that compartment 2 will exhibit both a first-order uptake and a first-order disappearance. The observed rate constant for uptake into compartment 2 will,

Table III. Kinetic Constants for TFN-d_7 in Rat Liver and Fat following Ip Injection at 500 μg/kg[a]

Constant	Value	Half-life, h
A	46 ± 7 ng/g	
B	4200 ± 3600 ng/g	
k_a	0.046 ± 0.008 h^{-1}	15 ± 2
k_b	0.023 ± 0.013 h^{-1}	31 ± 17

[a] Liver data were fit to the equation $[\text{TFN-}d_7]_{\text{liver}} = A\exp(-k_a t)$ and fat data were fit to the equation $[\text{TFN-}d_7]_{\text{fat}} = B[\exp(-k_b t) - \exp(-k_a t)]$.

Scheme III



moreover, correspond to that for disappearance from compartment 1.

By assuming liver to be representative of compartment 1 and fat to constitute compartment 2, the experimental data were fit simultaneously by computer to functions of the form of eq 1 and 2. The data points at each determination time were weighted according to the inverse of the observed variance. Curve fitting was done using the program MLAB, a modeling laboratory program from the National Institutes of Health Division of Computer Research and Technology. Computer-generated curves are shown in Figure 4, each point representing the average of two determinations on a particular animal. It is seen that the model appears to describe the data reasonably well.

The computer-estimated constants together with their standard errors are assembled in Table III. The half-life of TFN-d_7 in liver (15 h) is fairly precise (coefficient of variation 16%), since the experimental time scale covered about 5 half-lives for this tissue. On the other hand, the estimated terminal half-life for TFN-d_7 in fat (31 h) is uncertain (coefficient of variation 56%), since the experiments were continued for only 2 half-lives in this case, and there was a large variation in the concentrations measured in fat. To determine the persistence of TFN-d_7 in fat with greater precision would require extending the analysis over a much longer time period than 72 h.

More complicated models than depicted in Scheme II could account for the data. For example, by making the assumption that trifluralin is both released and absorbed by the tissues of compartment 1 throughout the duration of the experiment, a modified model such as shown in Scheme III would be obtained.

This model would require that the disappearance of TFN from liver be described by two exponentials instead of one, and that the terminal half-life of TFN-d_7 in liver would equal that found in fat. This model is physiologically more realistic than the preceding simpler model, but the variance of the data is evidently too large to require the inclusion of a second exponential term in the rate equation for liver (Figure 4). Thus, fitting of the data to more complex models such as Scheme III has not been attempted.

Because TFN-d_7 rather than unlabeled TFN was administered to the animals in these experiments, and because N-dealkylation is an important metabolic route (Emmerson and Anderson, 1966), it is pertinent to consider whether a deuterium isotope effect may have arisen in the metabolism of the compound. Two previous studies of N-demethylation reactions catalyzed by rat liver microsomes in which N–CD_3 compounds were used as substrates

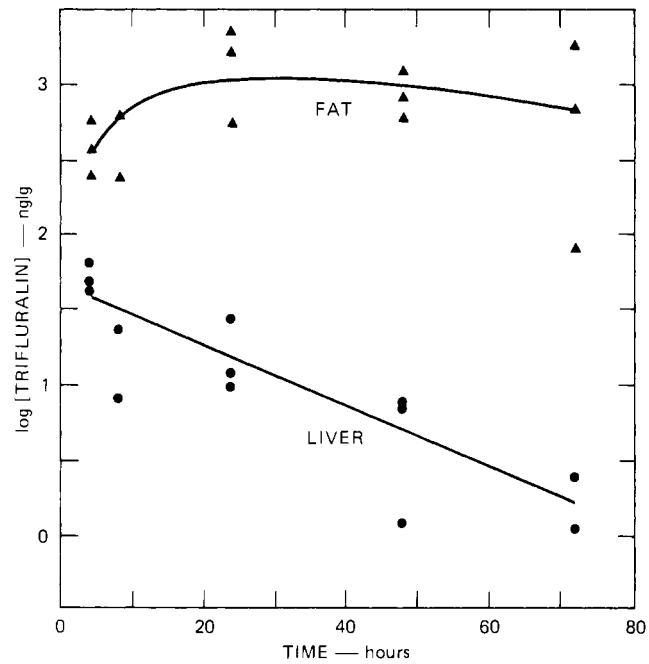


Figure 4. Simultaneous computer fits of TFN-d_7 concentrations in liver and fat of rats following intraperitoneal injection (500 μg/kg). Each value represents the average of two determinations on the tissue of a particular animal. Liver data were fit to the equation $[\text{TFN-}d_7]_{\text{liver}} = A\exp(-k_a t)$ and fat data were fit to the equation $[\text{TFN-}d_7]_{\text{fat}} = B[\exp(-k_b t) - \exp(-k_a t)]$.

have shown normal isotope effects ($k^H/k^D \simeq 2$) under substrate-limiting conditions (Elison et al., 1963; Dagani and Archer, 1976). Isotope effects in N-demethylations in vivo have also been demonstrated (Horning et al., 1975).

From these experiments, we may postulate that the N-dealkylation of a deuterated propyl chain of TFN-d_7 might exhibit a similar isotope effect. Since only one of the two alkyl chains was labeled in TFN-d_7, however, the overall isotope effect for metabolism via N-dealkylation will be smaller. Other metabolic transformations, such as reduction of the nitro groups (Emmerson and Anderson, 1966), as well as excretion and fecal elimination, are not expected to exhibit large isotope effects.

Although an isotope effect for N-dealkylation is likely and may reduce somewhat the half-life of unlabeled TFN in liver in comparison to that of TFN-d_7, the phenomenon is relatively unimportant from a toxicological standpoint. Extrapolations of pharmacokinetic data obtained with animal models to man are semiquantitative at best, even when the metabolic pathways are similar (Djerassi, 1970). The minor effect of using a deuterated analogue should not alter these conclusions.

Oral Administration. The detection of only small amounts of TFN-d_7 in fat and feces following chronic oral administration of the herbicide is in excellent qualitative agreement with the observations of Emmerson and Anderson (1966). These authors administered ^{14}C-labeled TFN orally for 14 days to rats at a much higher dosage level (100 mg kg^{-1} day^{-1}) than was used here. In their experiments, less than 8% of an oral dose was recovered as unchanged TFN in feces, but 70% of the radioactivity was eliminated in feces as metabolites, and urinary excretion (also as metabolites) accounted for most of the remainder. They found only low levels of TFN in fat, following chronic oral toxicity studies.

To explain these results, Emmerson and Anderson (1966) postulated that TFN is poorly absorbed from and extensively metabolized within the gut, presumably by microorganisms. Such a hypothesis provides a reasonable

explanation for the present findings. The oral route of administration evidently results in relatively little systemic absorption of the unchanged herbicide. The ip studies indicate, however, that once absorbed, the compound may persist in fat for lengthy periods of time.

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Extraction and Identification of the Major Metabolite of [*carbonyl*-^{14}C]Methabenzthiazuron after Degradation in the Soil

Werner Mittelstaedt,* Gerald G. Still, Hans Dürbeck, and Fritz Führ

After soil decomposition of [*carbonyl*-^{14}C]methabenzthiazuron, the metabolite fraction was extracted and separated from the unaltered methabenzthiazuron. Previous studies showed that due to small turnover rates, only limited concentrations of metabolites could be expected. Using HPLC, it was possible to isolate, concentrate, and purify the major metabolite. Its chromatographic properties were identical with 1-methyl-1-(2-benzthiazolyl)urea. The characterization was confirmed by mass spectroscopy fragmentation which yielded a parent ion at m/e 207. The manifold steps of separation and purification lead to relatively high losses. After separation from methabenzthiazuron, the metabolite fraction represented 2.4% of the applied radioactivity. After fractionation on μ-Porasil, only 0.4% was available for MS analysis as major metabolite. About 0.9% was separated and not identified, and 1.1% was used for TLC and LSC counting or lost during evaporation and redissolving, respectively.

The urea derivative methabenzthiazuron [1,3-dimethyl-3-(2-benzthiazolyl)urea] is a very effective herbicide in grain and certain vegetable crops (Hack, 1969; Kolbe and Zimmer, 1970). In lysimeter studies, under field conditions, using [*phenyl*-U-^{14}C]methabenzthiazuron, 83% of the applied radioactivity was recovered in soil 111 days after spray application to spring wheat (Führ, 1975; Führ and Mittelstaedt, 1976). The rate of methabenzthiazuron metabolism was very slow. At harvest, 90% of the chloroform extractable radiocarbon was shown to be unchanged herbicide. In addition to methabenzthiazuron, thin-layer chromatography resolved four other radiolabeled components from this soil extract (Table I). Even after 16 months, the metabolite fraction did not exceed 10% of

Radioagronomy Working Group, Kernforschungsanlage Jülich GmbH, D-5170 Jülich, Federal Republic of Germany (W. M., F. F.), Metabolism and Radiation Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Fargo, North Dakota (G.G.S.), Institute of Chemistry IV, Kernforschungsanlage Jülich GmbH, D-5170 Jülich, Federal Republic of Germany (H.D.).