# Effects of *N,N*-Diallyl-2,2-dichloroacetamide on Ethyl *N,N*-Di-*n*-propylthiocarbamate Uptake and Metabolism by Corn Seedlings

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In nutrient solution culture *N,N*-diallyl-2,2-dichloroacetamide (R-25788) at a concentration of $10^{-5}$ *M* protected corn from injury by EPTC (ethyl *N,N*-di-*n*-propylthiocarbamate) at rates ranging from $5 \times 10^{-5}$ to $10^{-4}$ *M*. R-25788 did not reduce EPTC uptake or alter EPTC distribution in the plants. Extracts from plants treated with [$^{14}$C]EPTC plus R-25788 contained less unaltered EPTC than plants which received no R-25788. In R-25788 plus [$^{14}$C]EPTC treated corn seedlings more $^{14}CO_2$ and [$^{14}$C]EPTC vapors were released than from seedlings treated with [$^{14}$C]EPTC alone. While these effects of R-25788 on EPTC metabolism and elimination from the plants could contribute to the reduction in EPTC injury, their magnitude did not seem adequate to explain the full mode of action of R-25788 as an EPTC antidote.

It has been shown that *N,N*-diallyl-2,2-dichloroacetamide (R-25788) provided protection to corn from EPTC (ethyl *N,N*-di-n-propylthiocarbamate) injury with no loss in activity of the herbicide on several weed species (Chang *et al.*, 1972; Meggitt *et al.*, 1972; Rains and Fletchall, 1971). It was effective when used as a seed treatment, as a tank mixed spray with EPTC and incorporated into the soil, or incorporated into the solution of a nutrient culture (Chang *et al.*, 1972). When used as a seed treatment it was more effective than 1,8-naphthalic anhydride (Chang *et al.*, 1973b), the first EPTC antidote reported for corn (Burnside *et al.*, 1971; Hoffmann, 1969). R-25788 also reduced corn injury from a number of other herbicides containing a carbamyl group (Chang *et al.*, 1973a).

The mechanism for the antidote action of R-25788 is not known. Since differential uptake or metabolism of a herbicide is often involved in its selectivity, it is possible that R-25788 protects corn from EPTC injury by altering these physiological processes in the plants. The objective of this study was to determine whether R-25788 affected the uptake, distribution, and/or metabolism of EPTC in corn plants.

## MATERIALS AND METHODS

**Sequence of EPTC and R-25788 Application.** Corn (*Zea mays* L. United Hybrid 106) seeds were germinated in moist paper towels for 3 days. Just before the first leaf emerged from the coleoptile, two seedlings were transferred into each 250-ml beaker which contained 200 ml of Hoagland's nutrient solution. The nutrient solution was changed every 2 days and the plants were maintained in a growth room with a 16-hr photoperiod with 11,000-lx light intensity at 22° and an 8-hr dark period at 18°. Four days later, when the plants were in the two-leaf stage and approximately 14 cm tall, EPTC was added to the nutrient solution at a concentration of $10^{-4}$ *M* for a duration of 2 days. R-25788 at a concentration of $10^{-5}$ *M* was added to the nutrient solution for a duration of 2 days at different times: (1) 2 days prior to EPTC application, (2) at the same time as EPTC treatment, and (3) 2 days after EPTC application. After each 2-day treatment period, the plants and the containers were rinsed with running water for 30 min before changing the nutrient solution. Shoots and roots of the plants were harvested separately 2 weeks after EPTC treatment. Fresh weights of the plant parts were expressed as a percentage of control plants which were not treated with the herbicide. Two plants in a beaker formed a sample unit and a randomized, complete block design with six replicates per treatment was employed.

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**Treatment with [$^{14}$C]EPTC.** [1-$^{14}$C]EPTC (1.33 Ci/mol, Stauffer Chemical Co., Richmond, Calif.) was used in the translocation and metabolism experiments. Corn seedlings were grown in 100-ml beakers with Hoagland's nutrient solution, two plants per beaker. When the plants were in the two-leaf stage, they were treated with 0.8 $\mu$Ci of [$^{14}$C]EPTC in 50 ml of nutrient solution with or without R-25788 at $10^{-5}$ *M*. The concentration of the herbicide was adjusted to $5 \times 10^{-5}$ *M* by adding unlabeled EPTC to the solution. The beakers were covered with a layer of parafilm to reduce vapor loss of the herbicide from the solution. Controls were prepared with 50 ml of the treatment solution without plants and sealed similarly. The plants were harvested 1, 3, 5, and 7 days after treatment, and separated into shoots, roots, and remaining corn seeds. At each harvest time, aliquots from the treatment solutions were assayed for radioactivity by liquid scintillation counting. The net loss of radioactivity, which was obtained by deducting the loss in the control beakers from the total loss in beakers containing the plants, was taken as the total [$^{14}$C]EPTC uptake by the plants. Each treatment had three replications and a randomized, complete block design was used.

**Analysis of Plant Extracts.** The plant parts were homogenized and extracted four times with 50% methanol, and, for each extraction, the homogenates were centrifuged at 1000*g* for 10 min. Aliquots of the supernatants were used directly for radioassay. Vacuum drying or lyophilization of the extracts was avoided because it could cause a severe vapor loss of EPTC and EPTC metabolites. Radioactivity in the pelleted residues was quantitated by combustion in a Packard 305 sample oxidizer and subsequent liquid scintillation counting of the liberated $^{14}CO_2$. [$^{14}$C]EPTC and its metabolites in the extracts were separated by partition with *n*-hexane. Radioactivity in the hexane fraction was found to be unaltered [$^{14}$C]EPTC while the radioactivity remaining in the aqueous phase was attributed to its metabolites.

Radiolabeled metabolites in the aqueous phase were analyzed by thin-layer chromatography (tlc) on silica gel G in butanol–acetic acid–water (4:1:1) and benzene–ethanol (75:25). Radioactive compounds on the tlc plates were detected by autoradiography and subsequent counting of the radioactivity in each spot.

**$^{14}CO_2$ and [$^{14}$C]EPTC Vapor Trapping.** To study the effects of R-25788 on vapor loss of EPTC and $^{14}CO_2$ from corn plants, the seedlings were grown in 50-ml erlenmeyer flasks containing 20 ml of nutrient solution. Treatments were made by replacing the nutrient solution with fresh nutrient solution containing EPTC at a concentration of $5 \times 10^{-5}$ *M* which contained 986,000 dpm of [$^{14}$C]EPTC. Concentrations of R-25788 in the treatment solution were 0 or $10^{-5}$ *M*. The flasks were sealed with a plug of para-

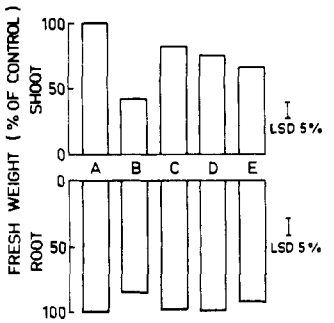


**Figure 1.** Phytotoxicity of $10^{-4}$ *M* EPTC to corn under the influence of $10^{-5}$ *M* R-25788 applied to the nutrient solution at different times for a period of 2 days: (A) control; (B) EPTC alone; (C) R-25788 and EPTC applied at the same time; (D) R-25788 applied 2 days prior to EPTC; (E) R-25788 applied 2 days after EPTC treatment.

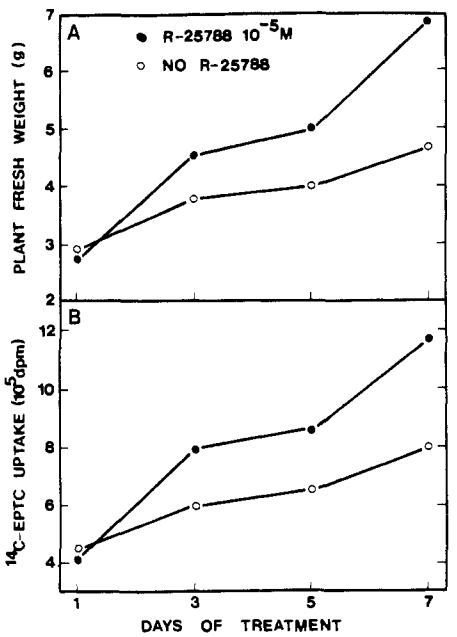


**Figure 2.** Effects of R-25788 on EPTC phytotoxicity to corn (A) and [$^{14}$C]EPTC uptake by corn plants (B) in nutrient solution culture.

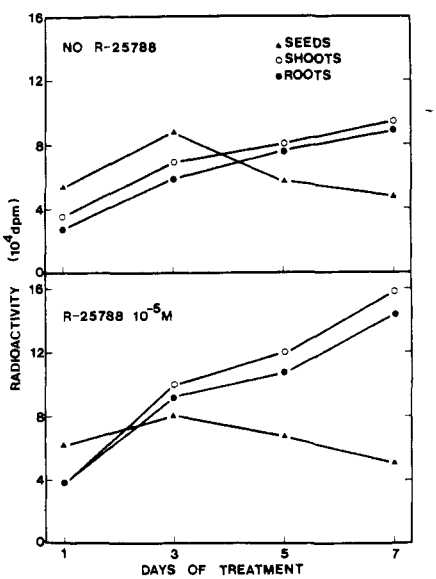


**Figure 3.** Distribution of radioactivity from [$^{14}$C]EPTC in corn seeds, roots, and shoots in nutrient solution culture with or without R-25788.

film and plasticine around the stem of the plants and wrapped with a layer of tin foil. The flasks were placed in Mason jars. Glass tubes were connected into the metal tops of the jars to provide an air inlet and outlet. To the outlet tube, four gas washing bottles were connected in series with fitting glasses. Tygon or rubber tubing could not be used because they showed a high affinity for EPTC. Two gas washing bottles were filled with 200 ml of 0.5 *N* potassium hydroxide as a trapping solution for $^{14}CO_2$ and two bottles in an ice bath were filled with 200 ml of *n*-hexane for the trapping of [$^{14}$C]EPTC (Nalewaja *et al.*, 1964). Filtered air was drawn to pass through a calcium chloride moisture trap into the Mason jars and was bubbled through the potassium hydroxide trap and then the hexane trap at a rate of 300 ml/min for 60 hr. At the end of the experiment, aliquots of the trapping solutions were removed for radioassay. The nutrient solutions were also assayed for radioactivity to determine the total loss of [$^{14}$C]EPTC from the closed system. The parafilm and plasticine plug also contained some radioactivity which was attributed to vapor loss of the herbicide from the treatment solution. The plants were extracted and assayed for total radioactivity as well.

## RESULTS

**Time Sequence Study of EPTC Uptake.** Application of $10^{-4}$ *M* EPTC in nutrient solution for a period of 2 days caused a severe reduction in corn shoot growth (Figure 1). The presence of $10^{-5}$ *M* R-25788 in the solution for a period of 2 days reduced the growth inhibition regardless of when it was applied. However, when R-25788 was applied 2 days after EPTC treatment (Figure 1E) the final plant growth was not as great as when the antidote treatment was made before or at the same time as the EPTC treatment (Figure 1C,D). Apparently, the antidote could prevent further injury from EPTC already taken up by the plants, but did not facilitate full recovery from injury which had already resulted from the EPTC pretreatment. EPTC treatment showed little effect on corn root growth (Figure 1).

**[$^{14}$C]EPTC Uptake.** In nutrient solution culture, R-25788 enhanced corn growth by reducing EPTC toxicity and the effect was significant as early as 3 days after treatment (Figure 2A). This protection effect of R-25788 was not due to an inhibition on EPTC uptake. In fact, R-25788 had no apparent effect on [$^{14}$C]EPTC uptake per unit plant weight. Total [$^{14}$C]EPTC uptake was greater with R-25788 treated plants (Figure 2B) and this increased uptake was proportional to the increased growth of these seedlings (Figure 2A,B).

**[$^{14}$C]EPTC Translocation.** The total radioactivity in the extracts and the residues was combined and the data were used to indicate the distribution or translocation of [$^{14}$C]EPTC and its metabolites in the plants (Figure 3). Here again, R-25788 had no effect on $^{14}$C distribution in the plants. The total radioactivity in shoots and roots of R-25788 treated plants was higher than in those of the nontreated plants. However, the concentration of $^{14}$C in terms of disintegrations per minute per unit weight of the seeds, roots, or shoots was not significantly affected by R-25788 treatment.

**[$^{14}$C]EPTC Metabolism.** Radioactivity in the residue after 50% methanol extraction was assumed to be metabolites of [$^{14}$C]EPTC, which increased with time of treatment from 13 to 35% of the total recovered activity in the roots, from 45 to 64% in the shoots, and from 1 to 4% in the seeds. R-25788 treatment had no effect on the percentage of these radioactivities. The seeds retained a much higher specific radioactivity than shoot or root, which was readily extractable by 50% methanol. In the methanol extracts from the seeds, the percentage of unaltered [$^{14}$C]EPTC decreased with treatment time from 87 to 43% of the total radioactivity recovered. Again, R-25788

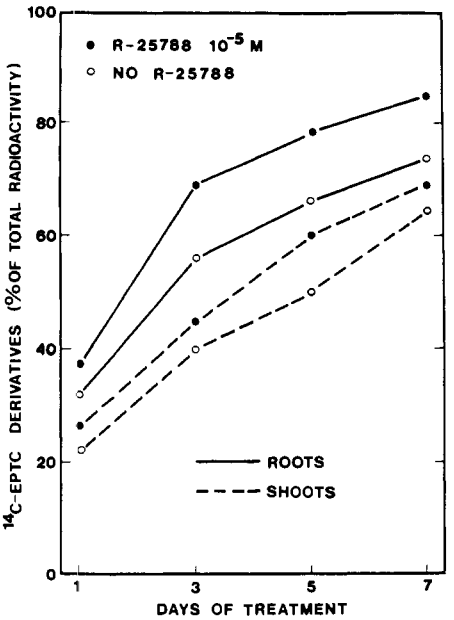


**Figure 4.** Influence of R-25788 on [$^{14}$C]EPTC metabolism in corn plants. Data show the metabolites of EPTC as a percentage of total radioactivity in a 50% methanol extract.

treatment showed no effect on the extent of [$^{14}$C]EPTC metabolism in the seed tissue. The only significant effect of R-25788 on [$^{14}$C]EPTC metabolism was observed in the proportion of unaltered [$^{14}$C]EPTC to its metabolites in the methanol extracts from shoots and roots (Figure 4). There was a higher proportion of [$^{14}$C]EPTC metabolites to unaltered [$^{14}$C]EPTC in both shoot and root tissues of the R-25788 treated plants than in those from the nontreated plants. Apparently R-25788 enhanced EPTC metabolism in these plant tissues.

Tlc analysis of the 50% methanol extractable derivatives of [$^{14}$C]EPTC indicated that R-25788 did not affect the quality of the metabolites in either the shoot or the root tissues (Table I). There were only minor differences in the percentages of the metabolites separated by the two solvent systems used. Metabolites from the seed tissues were not analyzed, but they should not have much influence on the antidote action of R-25788; in our preliminary observation it was found that removing the seeds from the seedlings before EPTC and R-25788 treatment did not alter the antidote effect of R-25788.

**$^{14}CO_2$ and [$^{14}$C]EPTC Vapor Loss from Shoots.** A substantial amount of radioactivity was detected in the potassium hydroxide solution (Table II) indicating that [$^{14}$C]EPTC was degraded to $^{14}CO_2$ by corn plants. Radioactivity in the hexane trap was identified by tlc as unaltered [$^{14}$C]EPTC. Since the flasks containing the roots and the herbicide solution were sealed, the [$^{14}$C]EPTC recovered in the hexane trap must have emanated from the shoot tissues outside the flask. The amounts of both [$^{14}$C]EPTC and $^{14}CO_2$ collected from the trapping system were higher for R-25788 treated plants than from plants which received no R-25788, and the increase was more than proportional to the enhanced growth of the plants. It appeared that R-25788 treatment enhanced both the degradation of [$^{14}$C]EPTC to $^{14}CO_2$ and the vapor loss of the herbicide from corn shoot tissues.

**Table I. Tlc Analysis of 50% Methanol Extractable [$^{14}$C]EPTC Metabolites from Corn Seedlings Grown for 3 Days in Nutrient Solution Containing [$^{14}$C]EPTC with or without $10^{-5}$ *M* R-25788**

| Tlc solvent[a] | Plant part | [R-25788], *M* | % radioactivity with $R_f$ | | | | |
|---|---|---|---|---|---|---|---|
| | | | 0 | 0.18 | 0.45 | 0.64 | 0.72 |
| A | Root | 0 | 6 | 19 | 30 | 36 | 9 |
| | Root | $10^{-5}$ | 7 | 20 | 28 | 37 | 8 |
| | Shoot | 0 | 2 | 16 | 24 | 44 | 14 |
| | Shoot | $10^{-5}$ | 4 | 16 | 29 | 33 | 18 |
| | | | $R_f$ | | | | |
| | | | 0 | 0.12 | 0.24 | 0.43 | 0.60 |
| B | Root | 0 | 67 | 10 | 10 | 6 | 7 |
| | Root | $10^{-5}$ | 70 | 10 | 10 | 7 | 3 |
| | Shoot | 0 | 57 | 14 | 9 | 11 | 9 |
| | Shoot | $10^{-5}$ | 59 | 15 | 8 | 8 | 10 |

[a] Solvent systems: (A) butanol–acetic acid–water (4:1:1); (B) benzene–ethanol (75:25).

**Table II. Loss of $^{14}CO_2$ and [$^{14}$C]EPTC Vapor from Shoots of Corn Seedlings Grown in Nutrient Solution Containing [$^{14}$C]EPTC (986,000 dpm) with or without R-25788[a]**

| Radioact. recovd as | Dpm for R-25788 concn of | |
|---|---|---|
| | 0 | $10^{-5}$ *M* |
| Treatment solution | 724,470 | 657,500 |
| [$^{14}$C]EPTC vapor | 21,445 | 32,522 |
| $^{14}CO_2$ | 10,200 | 16,097 |
| Plant tissue | 151,950 | 177,061 |
| Unrecoverable | 77,935 | 102,820 |

[a] Data represent the average from three experiments. Average fresh weights of corn plants at harvest time: 2.253 g in R-25788 treatment and 1.995 g in treatment without R-25788.

## DISCUSSION

When R-25788 was applied to corn seedlings after 2 days of pretreatment with EPTC in the nutrient solution, EPTC injury was significantly reduced. These results indicated that the antidote action of R-25788 was not confined to effects on the root uptake of EPTC. This observation was confirmed in studies with $^{14}$C-labeled EPTC, where R-25788 treatment actually resulted in a greater rate of EPTC uptake because of reduced EPTC injury and thus greater plant growth.

R-25788 did not interfere with the translocation of [$^{14}$C]EPTC in corn seedlings. However, extracts from plants treated with [$^{14}$C]EPTC plus R-25788 contained less unaltered [$^{14}$C]EPTC than corn seedlings which received no R-25788. Also, in R-25788 plus [$^{14}$C]EPTC treated corn seedlings more $^{14}CO_2$ and [$^{14}$C]EPTC vapor were released than from seedlings treated with [$^{14}$C]EPTC alone. The greater release of $^{14}CO_2$ might also be a reflection of the faster rate of [$^{14}$C]EPTC metabolism. These apparent effects of R-25788 on EPTC metabolism and elimination from corn plants could contribute to the observed reduction in EPTC injury. However, the magnitudes of these effects do not seem adequate to explain the full mode of action of R-25788 as an EPTC antidote. Moreover, these effects could simply be a result of reduced EPTC injury to the plant.

Clearly, a more definitive knowledge of R-25788 as an EPTC antidote will require more thorough knowledge of the mode of action of EPTC as a herbicide.

## ACKNOWLEDGMENT

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# Distribution of Mercury among Components of Eggs following the Administration of Methylmercuric Chloride to Chickens

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The mercury concentration of eggs produced by hens fed 10 ppm of $CH_3HgCl$ for 10 days increased sharply through 12 days of the experiment and then declined slowly for the subsequent 58 days. Maximum concentrations of more than 10 and 5 ppm of Hg were attained in whites and yolks of eggs, respectively. A similar pattern of response was observed with regard to radioactivity in eggs after hens were given 25 $\mu$Ci of $CH_3{}^{203}HgCl$ by a single i.p. injection. During the 70-day trial, hens fed $CH_3HgCl$ deposited 55% of the total Hg consumed in eggs, while eggs produced by hens given $CH_3{}^{203}HgCl$ contained 65% of the total radioactivity administered. Mercury was found in whites, yolks, and shells of eggs, with more than 80% of the total egg Hg occurring in the white. Additional research showed that the majority of the Hg in egg white was associated with the protein, ovalbumin, rather than with ovotransferrin, ovoglobulin, or ovomucoid.

Considerable information has been accumulated about the toxicity of various mercury-containing compounds for animals of the Aves class (Swensson and Ulfvarson, 1969; Fimreite and Karstad, 1971; Gardiner, 1972; Gardiner *et al.*, 1971; and Spann *et al.*, 1972). Concurrently, data have been presented describing the distribution of mercury among tissues of birds following the administration of mercury (Hg) in various chemical forms (Swensson and Ulfvarson, 1969; Gardiner *et al.*, 1971; Hough and Zabik, 1972; and Wright *et al.*, 1973). It has also been reported that mercury was transferred readily from the diet into eggs, particularly when alkylmercury compounds were fed (Tejning and Vesterberg, 1964; Kiwimäe *et al.*, 1969; Westöö, 1969; Fimreite *et al.*, 1970; Campbell *et al.*, 1971; and Spann *et al.*, 1972).

The data presented by Smart and Lloyd (1963), Kiwimäe *et al.* (1969), and Campbell *et al.* (1971) indicated that the majority of mercury in eggs was present in the white when alkylmercury compounds were fed. The research described herein was conducted to determine the distribution of mercury among the white, yolk, and shell of eggs following the administration of methylmercuric chloride to chickens. Research was also conducted to determine the protein fractions of egg white with which mercury was predominately associated.

## REAGENTS

Reagent grade methylmercuric chloride was obtained from Alfa Inorganics Ventron, Beverly, Mass. [$^{203}$Hg]Methylmercuric chloride (specific activity, 130 mCi/g; radiopurity, in excess of 95%) was purchased from Amersham-Searle Corp., Arlington Heights, Ill. The purified proteins of egg white, ovalbumin, ovotransferrin, ovoglobulins, and ovomucoid were obtained from Sigma Chemical Co., St. Louis, Mo., and the Sephadex G-10 from Pharmacia Fine Chemicals Inc., Piscataway, N. J.

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## APPARATUS

A Model 305 atomic absorption spectrophotometer (Perkin-Elmer Corp., Norwalk, Conn.) was used in the determination of mercury. Radioactivity was determined with a Model 1085 deep-well $\gamma$ counter equipped with a NaI (thallium activated) crystal (Nuclear-Chicago, Chicago, Ill.). Ultrafiltration was performed with a Model 12 filtration cell (Amicon Corp., Lexington, Mass.). A Gilson Model MF fraction collector (Gilson Medical Electronics, Middleton, Wis.) was used during gel filtration, and electrophoresis was done using a Canalco Model 6 apparatus (Canalco, Rockville, Md.).

## PROCEDURE

Five Single Comb White Leghorn hens, approximately 68 weeks of age and kept in individual metabolism cages, were used. Three hens were fed a practical ration in which methylmercuric chloride ($CH_3HgCl$) was included at a level of 10 ppm. This level of $CH_3HgCl$ was shown by analysis to correspond to about 8 ppm of Hg. The $CH_3HgCl$ was dissolved in 95% ethanol prior to mixing in the ration. The mercury-containing ration was fed *ad libitum* for 10 consecutive days. The hens were then fed a ration which contained no added mercury for the remainder of the 70-day trial.

Each of the remaining two hens, also fed the practical laying hen ration, were given a single, intraperitoneal injection of 25 $\mu$Ci of radioactivity as [$^{203}$Hg]methylmercuric chloride. The carrier solution was 0.1 ml of ethanol.

Eggs were collected from all hens during the 70-day trial and were weighed. The eggs were broken and the white, yolk, and shell were separated. The whites and yolks from eggs produced by hens fed 10 ppm of $CH_3HgCl$ were analyzed for total Hg by atomic absorption spectrophotometry according to the method of Deitz *et al.* (1973). Radioactivity of the components of eggs obtained from the hens injected with $CH_3{}^{203}HgCl$ was determined using the deep-well, $\gamma$ counter. The volume and geometry of the samples were standardized to 1.0 cm$^3$ and compared with