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AN ALBERTA FARM DUGOUT**

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NWRI Contribution No. 92-62

**FATE OF TRIFLURALIN IN ANAEROBIC SEDIMENT
FROM AN ALBERTA FARM DUGOUT**

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MANAGEMENT PERSPECTIVE

Dugouts are commonly the only source of water on prairie farms. Treflan (trifluralin) is used extensively on many of these farms. A significant possibility of contamination of such dugouts from nearby application of Treflan or from long range atmospheric transport (observed in other studies) exists. This laboratory study established that 95% of trifluralin added to dugout sediments disappeared in two days but two persistent degradation products appeared and were identified. All three compounds were subsequently found in both sediments and leeches from two Alberta farm dugouts more than a year after experimental treatment with Treflan. A food chain uptake route for trifluralin and the potentially toxic degradation products is thus suggested. This study will help to define the hazard potential of TRF contaminated dugouts to farm stock, humans and wildlife. Further studies are suggested to determine the presently unknown toxicity of the stable degradation products.

SOMMAIRE À L'INTENTION DE LA DIRECTION

Il est courant que les étangs artificiels soient la seule source d'eau sur les fermes situées dans les provinces des Prairies. Le Tréflan (trifluraline) est un herbicide largement utilisé dans beaucoup de ces fermes. Il existe une réelle possibilité de contamination de ces étangs par suite de l'application de tréflan à proximité des étangs ou sous l'effet du transport atmosphérique de longue distance (fait documenté ailleurs). La présente étude de laboratoire a établi que 95 % de la trifluraline ajoutée à des sédiments prélevés dans des étangs artificiels, disparaissait en deux jours, mais que deux produits de décomposition persistants apparaissaient. Ils ont été identifiés. Les trois composés ont été trouvés dans les sédiments de deux étangs artificiels de l'Alberta ainsi que dans des sangsues qui y ont été capturées à plus d'un an après le traitement expérimental au tréflan. Les auteurs proposent un cheminement possible, dans la chaîne alimentaire, de la trifluraline et des produits de dégradation potentiellement toxiques. Cette étude aidera à définir les risques potentiels que les étangs artificiels contaminés à la TRF font subir aux personnes, aux animaux d'élevage et à la faune. De nouvelles études sont jugées nécessaires afin de déterminer la toxicité inconnue jusqu'à maintenant des produits stables de décomposition.

ABSTRACT

Anaerobic surficial sediment from a farm dugout in central Alberta was treated with sufficient Treflan^{TM 3} herbicide added to yield 5 µg/g dry weight trifluralin (TRF) and maintained in the laboratory under anaerobic conditions. Samples collected at intervals over 32 days were analysed for trifluralin and related compounds. More than 95% of the trifluralin disappeared within two days but levels then stabilized and even increased slightly, suggesting regeneration from an initially unextractable form. A product of reductive transformation, α,α,α -trifluoro-5-nitro-N⁴,N⁴-dipropyltoluene-3,4-diamine (R1TRF), was identified. R1TRF was found from day 0, reached a maximum of 0.3 µg/g, then slowly declined over the duration of the study. A second reductive transformation product, α,α,α -trifluoro-N⁴,N⁴-dipropyltoluene-3,4,5-triamine (R2TRF), was also identified. This compound appeared transiently in low concentrations between day 2 and day 16 of the study. The findings of this study were used to explain the bioaccumulation of TRF and related compounds in leeches from a Treflan treated dugout.

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RÉSUMÉ

Les sédiments anaérobies de surface prélevés dans un étang artificiel d'une ferme située dans le centre de l'Alberta, ont été traités avec l'herbicide tréflan^{md 1} en quantité suffisante pour donner 5 ug.g⁻¹ (poids sec de trifluraline) (TRF); les sédiments ont été gardés en milieu anaérobie au laboratoire. La trifluraline et des composés apparentés ont été dosés dans des échantillons prélevés à différents intervalles pendant 32 jours. Plus de 95 % de la trifluraline avait disparu au bout de deux jours, mais la concentration s'est ensuite stabilisée et même légèrement accrue; cela donne à penser qu'il se produit une régénération à partir d'une forme qui était initialement inextractible. Un produit de transformation par réduction, l' $\alpha\alpha\alpha$ -trifluoro-5-nitro-N⁴,N⁴-dipropyltoluène-3,4-diamine (R1TRF), a été identifié. Le RETRF a été trouvé dès le jour 0, a atteint un maximum de 0,3 ug.g⁻¹ pour que sa concentration diminue ensuite jusqu'à la fin de l'étude. Un deuxième produit de transformation par réduction, l' $\alpha\alpha\alpha$ -trifluoro-N⁴,N⁴-dipropyltoluène-3,4,5-triamine (R2TRF), a été aussi identifié. Ce composé est apparu de façon transitoire, et à faible concentration, entre le jour 2 et le jour 16 de l'étude. Les résultats de l'étude ont servi à expliquer la bioaccumulation de TRF et de ses composés apparentés dans des sangsues capturées dans un étang artificiel traité au tréflan.

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INTRODUCTION

Trifluralin (α,α,α -trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine), referred to as TRF in this paper, is a preemergent herbicide extensively used in the prairie provinces on a wide range of crops. The selective toxicity and persistence of trifluralin, when incorporated into soil, allows it to interrupt the growth processes of many weeds over a full season (OMAF 1989). Mammalian toxicity is reported to be low (Probst et al., 1975) while toxicity to fish is moderate to high, depending on the species (Macek et al., 1969; Fabacher and Chambers, 1974). Toxicity to freshwater benthic invertebrates is reported to be low to moderate with daphnia and seed shrimp showing the most sensitivity (Sanders and Cope, 1966; Sanders, 1970). Reported toxicity values are for solutions of TRF; however TRF has a rather low water solubility of about 0.1 - 0.3 mg/L (Probst et al., 1975) and has a strong affinity for soil organic matter (Savage, 1978). Francis et al. (1989) showed that TRF levels in runoff were directly related to the suspended sediment load of the runoff and accounted for up to 0.3% of the applied TRF.

The use of dugouts for water supplies in northern Alberta is widespread, due to the poor quality and supply of groundwater throughout much of the region. On many farms the dugouts are used for domestic water supplies in addition to livestock watering and general farm uses. Although the dugouts are usually located close to the farm buildings for convenience, crops are often grown almost to the margins, with little protection offered from contamination by the wide range of chemicals used in modern farming practise.

An earlier study (Murphy et al., 1990) examined the effect of liming on TRF added to two dugouts in the Peace River area of northern Alberta. At the conclusion of this study, residues of TRF and an unidentified related compound were found in the dugout surficial sediments. An incidental finding of the study was a significant bioaccumulation of TRF (BAF 1400) and the same related compound in

leeches collected from the dugouts. These compounds were detected in leeches at significant levels several weeks after TRF was undetectable in the water column. The body burden of these compounds observed in leeches was suspected to be due to persistent but available residues of TRF and the products of anaerobic degradation in the sediment.

The principal goal of this study was to determine the fate of TRF in dugout sediment under anaerobic conditions. A further goal was to identify any TRF degradation products formed and to investigate the hypothesis that the unidentified TRF related compound found in dugout leeches was acquired from the products of anaerobic reduction of TRF in the sediments.

PROCEDURES

Sediment Treatment

Approximately 1 kg of surficial sediment (0-10 cm) was collected from a farm dugout east of Edmonton in central Alberta. The sediment was returned to the laboratory and screened to remove large debris. The bulk sediment was then transferred to a stainless steel tray and thoroughly blended on a shaker at low speed. Three subsamples of approximately 30 g each were immediately removed and freeze dried in shallow glass dishes. Sufficient Treflan (the commercial trifluralin formulation) was added to the remainder of the bulk sediment to produce a sediment dry weight concentration of approximately 5 $\mu\text{g/g}$ trifluralin and the mixture again thoroughly blended on the shaker at low speed. Eighteen subsamples of approximately 30 g in 50 ml glass culture tubes with tightly sealed teflon-lined screw caps were placed in an incubator at 15 °C in the dark. Three tubes, designated T0, were immediately transferred to shallow glass dishes and freeze dried as described above. Subsequent samples, in sets of 3, were removed and freeze dried at days 2,

4, 16 and, the final set of the study, day 32. All the freeze-dried samples were placed in tightly sealed plastic vials and shipped to the laboratory for analysis.

Extraction

Extraction and cleanup of freeze-dried sediments were based on a scaled down modification of the method of Lee and Chau (1983). The dried sediment subsamples were ground to a fine powder with a glass pestle and mortar and a 1-6 g subsample was sonicated for 3 minutes after the addition of 100 ml acetone and 1 ml 1 M KOH. The resulting extract was filtered through prewashed Celite 545 and evaporated to 50 ml. The concentrated extract was added to 100ml of 2% KHCO₃ and extracted with dichloromethane (DCM). A solvent exchange was effected by rotary evaporation with hexane using iso-octane as a keeper.

Cleanup

The concentrated extract was cleaned up by elution through wide bore pasteur pipet mini columns containing 2 g 10% deactivated Florisil with 14 ml 25% toluene in hexane (Lee and Chau, 1983). The eluant was evaporated under a stream of dry nitrogen to a final volume of 10 ml or 1 ml, depending on the expected concentration range.

Analysis

Cleaned up sediment extracts were analysed by capillary gas chromatography-mass spectrometry on a Hewlett Packard 5970 gas chromatograph-mass spectrometer (GC-MS).

After an initial full scan examination of a concentrated solution containing all three compounds of interest (*vide infra*), the GC-MS was set to collect selected ions at m/z 335, 306, 305, 276, 275, and 246. A DB-5 column, 30 m x 0.25 mm ID was programmed from 50 °C to 260 °C at 4 °C/ min. TRF standard solutions of 73 and 292 pg/ μL in toluene were used for quantitation of TRF. In the absence of available standards for R1TRF and R2TRF, a separate analysis of the concentrated solution of TRF, R1TRF and R2TRF was made using a flame ionization detector (FID) and GC-MS calculation factors computed assuming equal FID response factors for each compound.

RESULTS AND DISCUSSION

Since standards of the products of TRF degradation were not available, and would possibly be produced in very small amounts in the treated dugout sediments, it was decided to attempt to produce sufficient quantities for identification by larger scale sediment treatment. Two hundred g of a local high organic carbon anaerobic sediment was treated with 100 mg TRF added in 3 equal increments at 2 day intervals. The mixture was sealed from the air and stored in the dark at 22 °C. After 6 days the sediment was extracted by a scaled up version of the procedure described above, and when analysed, was found to contain 2 compounds not previously present in addition to TRF in greatly reduced quantity. Sufficient amounts of the new compounds were present for identification by GC-MS.

The electron impact mass spectrum of trifluralin is uncomplicated, with a 335 m/z molecular ion of about 10% of the base peak at 306 m/z and a strong peak at 264 m/z as reported in other studies (Downer et al., 1976). The two compounds produced in the sediment exhibited mass spectra remarkably similar to that of trifluralin but reduced throughout by 30 and 60 m/z units respectively. The obvious, and only plausible, interpretation of these TRF related mass spectra is the sequential

reduction of the two nitro groups to amino groups. We chose to call the compound with one amino group R1TRF, and the compound with two amino groups R2TRF. Indirect support for this interpretation is found in the landmark study of Golab et al. (1979). He identified more than 40 degradation products of TRF in field soils and flooded field soils. The products R1TRF and R2TRF were dominant components in TRF treated flooded soils. The proposed structures are shown in Figure 1.

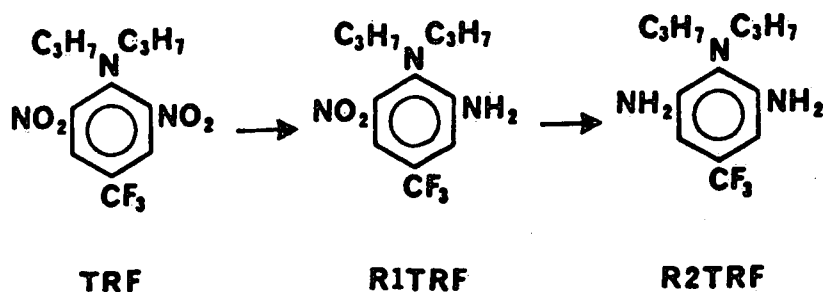


Figure 1. Proposed route of reductive transformation of trifluralin in anaerobic sediments.

The presence of detectable molecular ions (M^+) and strong peaks at $M^+ - 29$ m/z suggested the use of these ions for quantitative analysis, using the $M^+ - 29$ m/z ion for quantitation and the M^+ ion for confirmation of identity. Since only TRF was available in pure form for the preparation of quantitative standards, a separate analysis was made using a flame ionization detector against quantitative solutions of TRF. Relative response factors were calculated for the GCMS responses to R1TRF and R2TRF. The assumption made, of essentially equal response factors on the FID detector for each compound, is believed valid for closely related compounds.

TreflanTM is a solution of trifluralin in an unidentified carrier and stated to be 545 g/L by the manufacturer. The solution proved on analysis to be very close to that value at 539 g/L. The amount of Treflan added produced a mean concentration of 5.21 $\mu\text{g/g}$ measured immediately after addition and blending.

The concentrations of TRF, R1TRF and R2TRF observed over the 32 day duration of the experiment are shown graphically in Figures 2,3 and 4. The range of 1.05 $\mu\text{g/g}$ in the triplicate T0 samples suggested satisfactory, though not ideal, blending of the added TRF. The finding of a mean concentration 0.21 $\mu\text{g/g}$ of R1TRF at T0 was somewhat surprising as we had anticipated a biochemical route of anaerobic degradation. The logistical constraints of the procedures leads to a minimum of approximately 30 minutes between addition of the TRF and extraction of the T0 samples. This would be sufficient for chemical reduction to occur, but presumably not sufficient time for a significant amount of biologically mediated reduction.

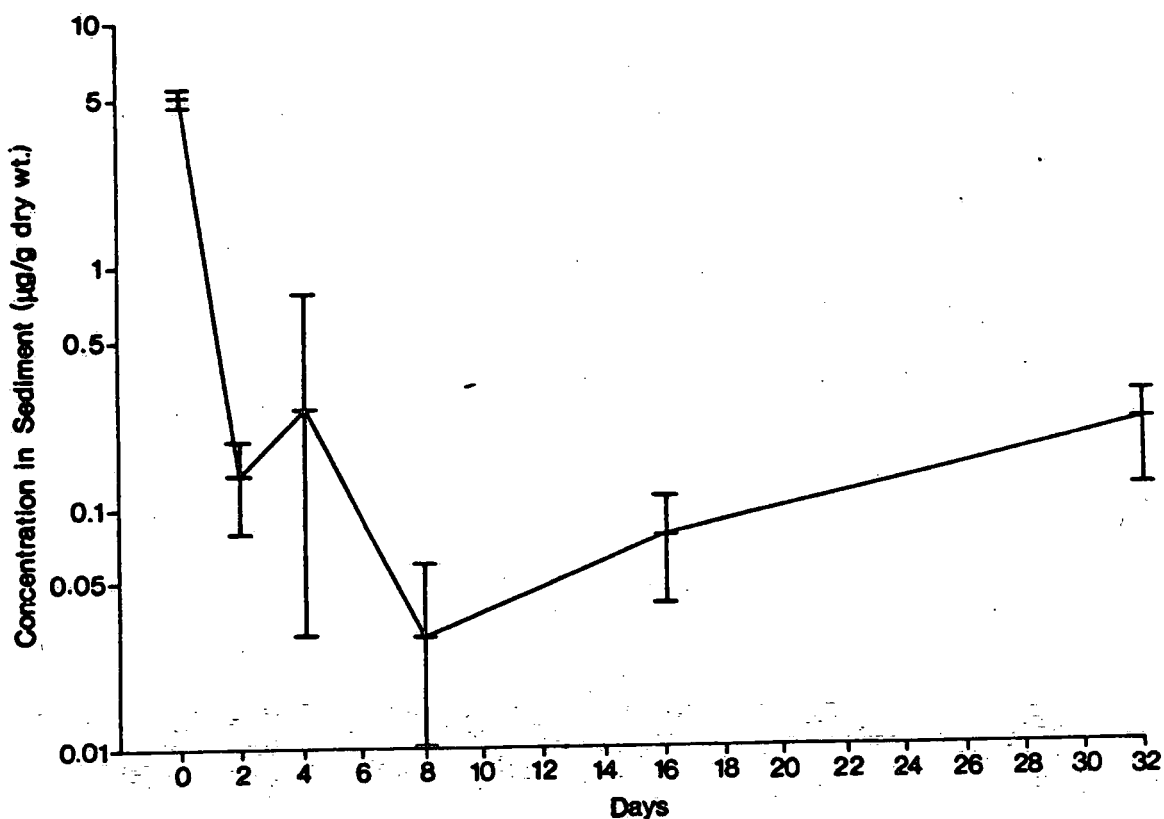


Figure 2. Concentration of TRF in treated dugout sediment. Vertical bars show range and mean of observed concentrations.

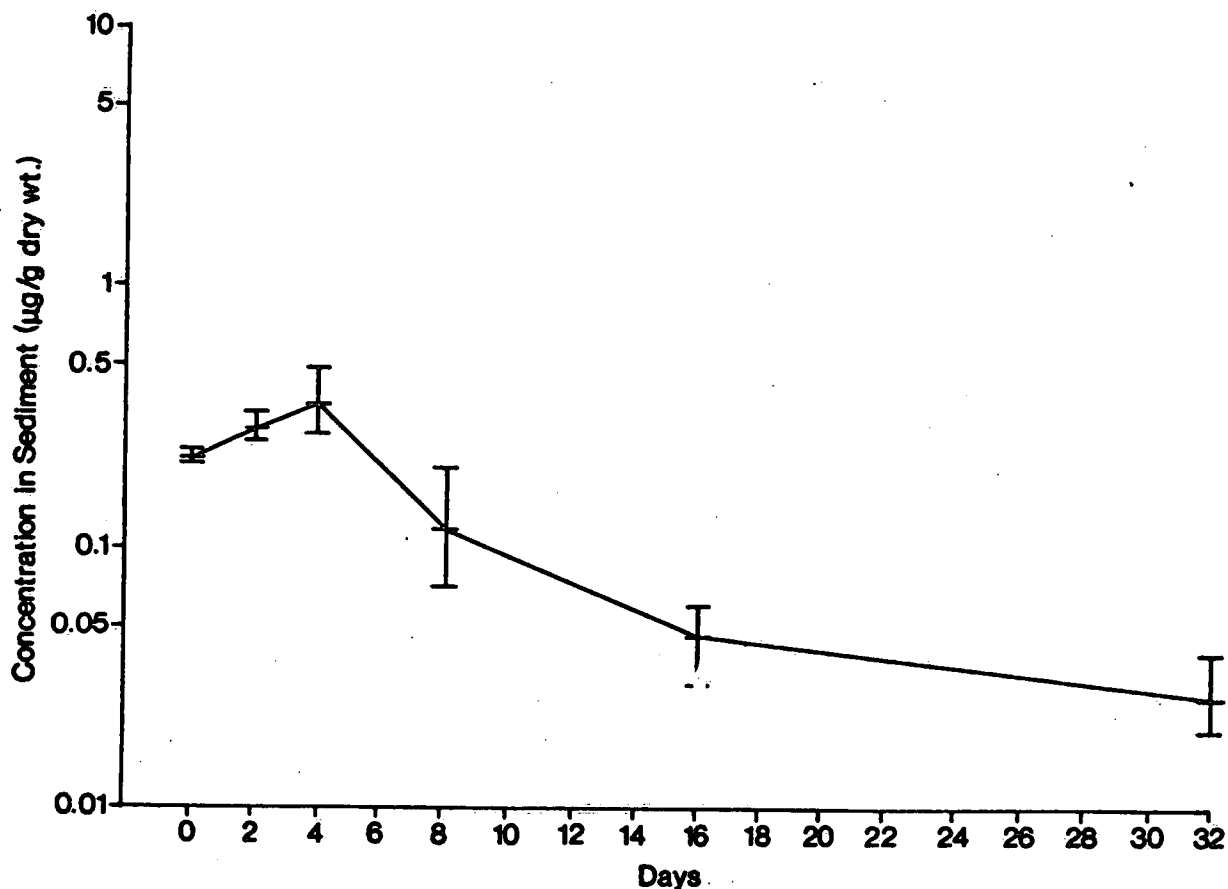


Figure 3. Concentration of R1TRF in treated dugout sediments. Vertical bars show range and mean of observed concentrations.

After only two days, the concentration of TRF remaining had fallen to about 3% of the added amount and less than 1% by day 8. Small increases were observed on days 16 and 32 with a final mean concentration of 0.23 $\mu\text{g/g}$ representing just under 5% of the added amount. The extent of TRF loss within the short time span of two days, and the concurrent, essentially immediate, appearance of R1TRF suggests a dominant chemical mechanism for the observed reduction. Sanders and Wolfe (1985) found a similar rapid initial disappearance in a laboratory study on model nitroaromatic compounds in anaerobic sediment water systems. They also found that heat and chemical sterilization of the sediment water mixture reduced but did not eliminate the disappearance rate constants of the model nitroaromatics.

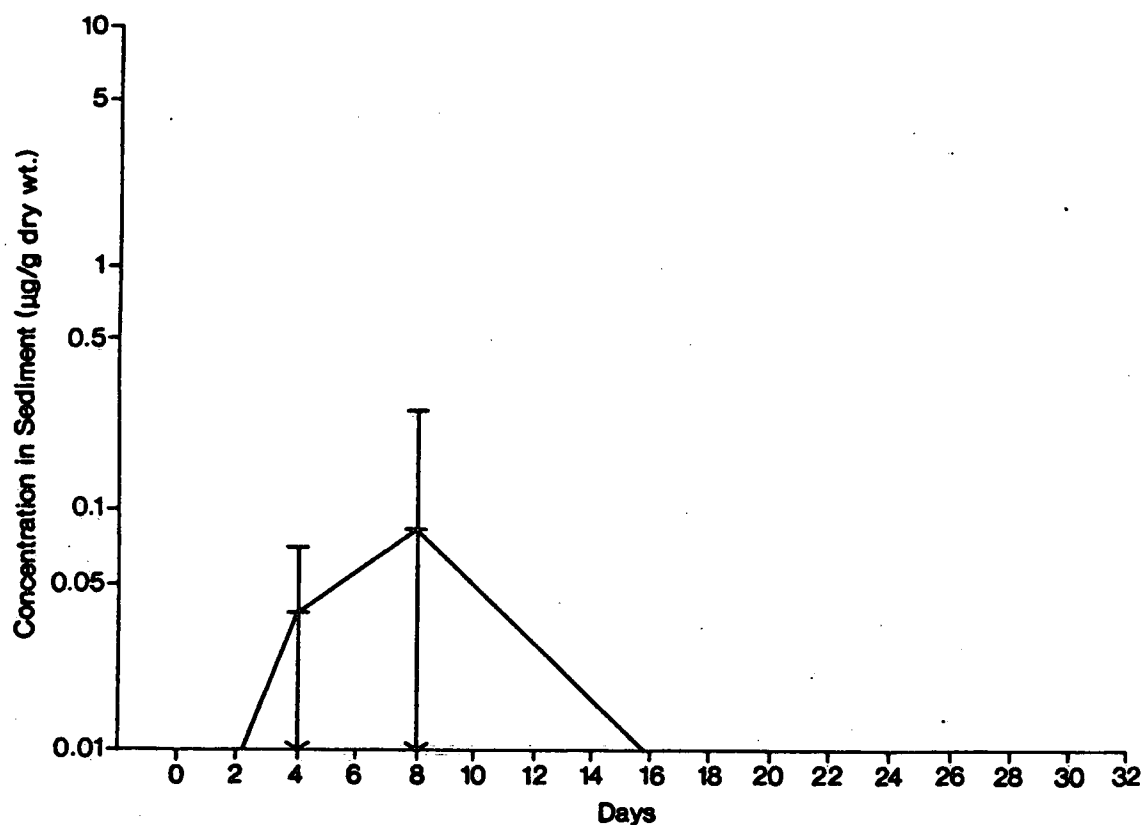


Figure 4. Concentration of R2TRF in treated dugout sediment. Vertical bars show range and mean of observed concentrations.

This supports our suggestion of abiotic processes for at least a part of the early losses. The increase in TRF concentration observed after day 8 is small, both in terms of the added amount and the range of triplicate observations, but appears to be real. Release of small amounts of TRF from an initially unextractable bound form, perhaps involving incorporation into biological organisms, is suggested. The experience of this analyst and others, with TRF spiked into biologically inert sediments, indicated that >90% of the TRF spike is recoverable by the analytical procedures employed.

After the immediate initial production of 0.22 $\mu\text{g/g}$ R1TRF, a small increase to a maximum of 0.37 $\mu\text{g/g}$ by day 4 was observed. This was followed by a gradual decline to 0.03 $\mu\text{g/g}$ at day 32. The second, fully reduced, transformation product R2TRF was not observed until day 4, and reached a maximum concentration of only 0.09 $\mu\text{g/g}$ at day 8. No detectable amounts of R2TRF were found after day 8. The significant lag time of four days before any observable production of R2TRF suggests dominant biochemical mechanisms for this product. Other studies (Golab and Amundson 1975; Golab et al. 1979) have indicated that these two reduction products are intermediates in the ultimate biochemical production of non-extractable metabolites.

The total concentration of TRF, R1TRF and R2TRF is virtually constant at about 5-6% of the added TRF after day 8. Complete mineralization of the remainder appears unlikely. Golab and Amundsen (1975) suggested that up to 50 % of the initial TRF may be transformed into sediment bound non-extractable residues.

The biphasic pattern of degradation observed in this study has been described elsewhere as typical of first order rate kinetics (Smith et al. 1988). The importance of the second, slower phase is emphasised in such descriptions. Although some 95% of the added TRF was lost or transformed to unextractable products in only two days, the environmental significance of the slow second phase is emphasized by the fact that R1TRF was significantly bioaccumulated in leeches collected from two northern Alberta farm dugouts 3-4 weeks after treatment with 0.5 mg/L TRF (Murphy et al. 1990). The ratio of TRF to R1TRF was approximately 3:1. No R2TRF was observed. In recent, not yet published, observations from the same dugouts, R1TRF and R2TRF were found in the both surficial sediments and the detritus feeding leech Nepheleopsis obscura collected a full year after the initial treatment. R2TRF was by far the predominant compound in both sediments and leeches. This is not readily explained by this short term study, where the R2TRF appeared only transiently. The

large dose treatment of local (Ontario) sediments for identification purposes in this study produced both R1TRF and R2TRF in 6 days at 22 °C, with R2TRF marginally predominating. The reduction processes observed in sediments from three widely separated localities suggest that very rapid initial losses or immobilization is followed by slow biochemical reduction mediated by commonly occurring microorganisms in the anaerobic sediments with R2TRF the eventual dominant component of the non bound TRF related residues. The finding of the same TRF related compounds, in similar relative proportions, in leeches as in the sediments, suggests that a food chain transfer of TRF compounds occurs from sediments through benthic organisms to leeches and possibly beyond to fishes and other predators. This long term persistence, and accumulation in the food chain, may be of significance to aquatic biota in water bodies receiving discrete or continuing TRF inputs, even at low levels.

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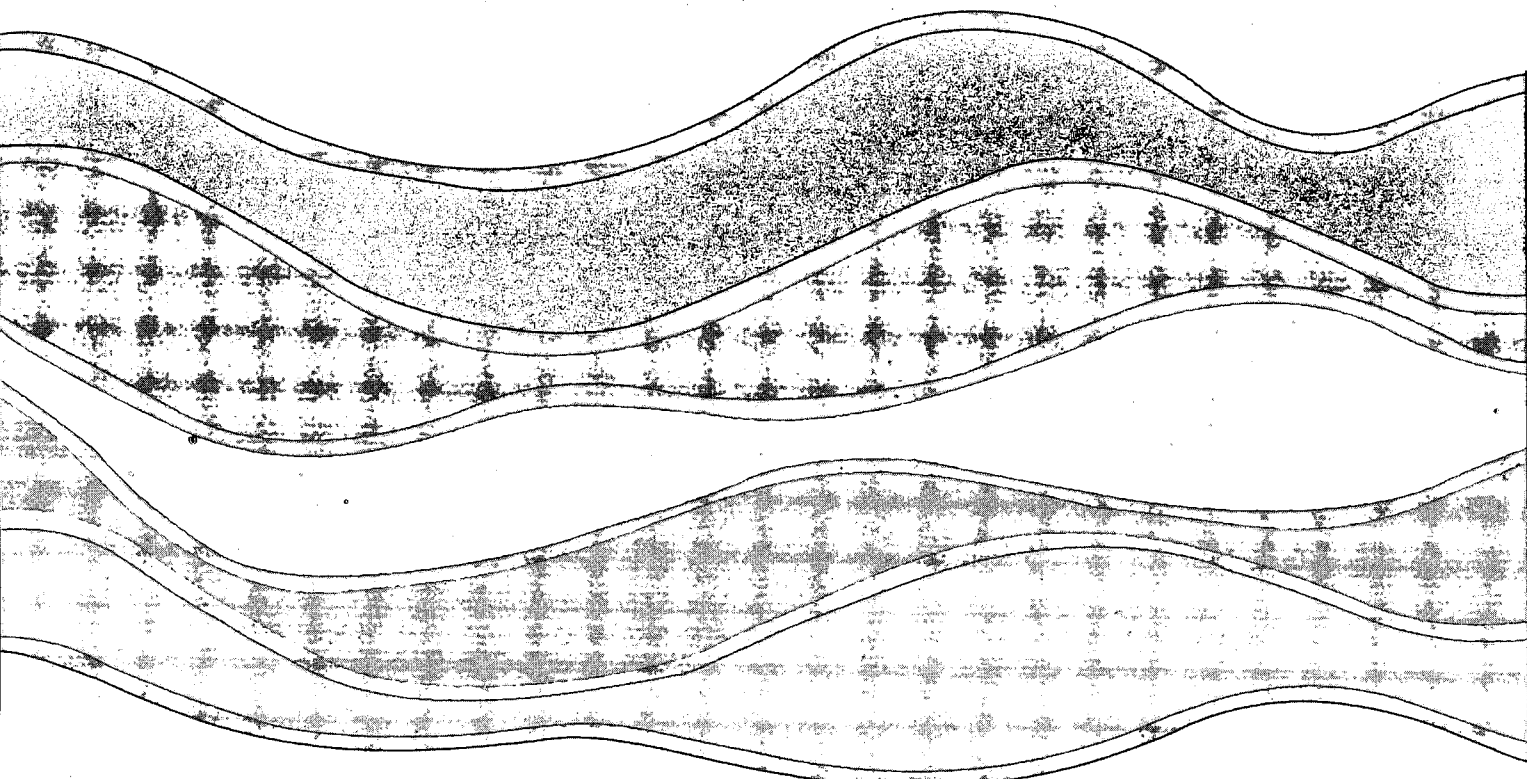
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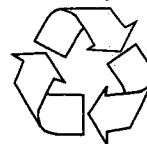
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