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Consumption and Degradation of ^{3}H-Polyethylene/Starch Disks by Terrestrial Isopods

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The recent development and commercial use of cornstarch and other additives in plastic formulations to promote degradability is potentially a valid approach for reducing the adverse impact of plastics in terrestrial and aquatic ecosystems. However, the rapid development of starch-based polyethylene plastics has exceeded our understanding of the biological properties of these compounds, such as whether these blended substances are actually more readily degraded and/or deteriorated than pure polyethylene (PE). In addition, the lack of standard tests and definitions regarding the fate of plastics in the environment has allowed the use of terms such as degradable by plastic manufacturers, while confusing consumers (Krupp and Jewell, 1992).

Wool (1988) has conducted degradation/metabolism tests with a variety of macroinvertebrates, including crickets, snails, slugs, cockroaches, and millipedes. Changes in film appearance, as well as fecal examinations were used to confirm the extent of film degradation. In general, starch/PE films containing less than 10% starch were not consumed. Furthermore, it has been shown that the degradation of starch/PE films by insects can involve three stages: mastication, digestion, and exo-corporeal degradation. The stages include processes (physical, chemical, and microbial) which contribute to the destruction of the film as the film is ingested, digested, and excreted (Sahlin, 1988; Wool et al., 1989; Wool and Goheen, 1990).

Recent studies in an aquatic environment indicated the potential importance of midge-fly larva (*Dicrotendipes* sp.) in biodegradation of plastic films containing starch (40%), PE, and polyethylene-co-acrylic acid (Imam et al., 1992).

Previously, we reported on the primary degradation of a variety of PE films containing different percentages of starch (0%-50%) and other additives (pro-oxidant, oxidized polyethylene) using earthworms (*Eisenia fetida*, *Lumbricus terrestris*, *Aporectodea trapezoides*, *Aporectodea tuberculata*), cockroaches (*Periplaneta americana*, *Blaberus* sp., *Blattella*

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germanica), termites (*Reticulotermes flavipes*), sowbugs (*Porcellio laevis*), and crickets (*Acheta domesticus*) (Tsao et al., 1993). Primary biodegradation was defined as a biologically produced deterioration of the film to the point where there was a loss of the initial physical properties. The results indicated that crickets, cockroaches, and sowbugs consumed starch-containing polyethylene films most readily. In addition, the degree to which the films were attacked and consumed was directly related to the starch content of the film. Films with oxidized polyethylene and those containing pro-oxidant were also consumed. None of the 4 species of earthworms tested, nor the termites showed any activity towards the starch/polyethylene films. The variety of organisms tested suggested that the results should have implications for both compost environments and discarded polymers. However, questions remained as to whether the PE in the films was chemically changed after consumption. Further studies of the degradation and degradation products of these blended substances was hampered by the difficulties inherent with chemical analysis of polymers.

Seminal research on PE degradation and the mechanisms involved was aided by the utilization of radiotracers. Using ^{14}C-labeled PE, Albertsson and coworkers (Albertsson, 1978; Albertsson et al., 1978; Albertsson and Banhidi, 1980; Albertsson et al., 1987) were able to detect the minute amounts of $^{14}CO_2$ being produced from biological degradation of the ^{14}C-PE, as well as propose a mechanism for PE degradation. To further study the degradation of starch/PE plastics by soil macroinvertebrates, we synthesized disks containing ^{3}H-labeled PE, starch, and oxidized PE and tested their degradability using the common soil isopod, *Armadilidium vulgare*. The results of those studies are reported here.

MATERIALS AND METHODS

The plastic disks used in the experiments were synthesized at Iowa State University. Initially, ^{3}H-poly-1,3-butadiene (dissolved in toluene) was purchased from Amersham Corporation (Arlington Heights, IL). The ^{3}H-poly-1,3-butadiene was reduced to ^{3}H-PE by refluxing with *p*-toluene-sulfonhydrazide in toluene with a N_2 atmosphere. NMR analyses were used to confirm complete reduction of the poly-1,3-butadiene to PE.

Percentages of ^{3}H-PE, oxidized PE (Aldrich, Milwaukee, WI), and starch (Aldrich, Milwaukee, WI) were determined by weight. The components were mixed in a round-bottom flask containing refluxing toluene to dissolve the PE. Contents of the flask were decanted into a 50 mL centrifuge tube containing methanol to precipitate the PE, causing the polymer to collapse around the starch granules. This sample was centrifuged for 10 minutes and the supernatant was removed. The precipitate was dried under N_2 and pressed into disks with an IR pellet press. The pressed disks were used without further cleanup. Specific activities of the disk samples were as follows: 100% ^{3}H-PE, 4.94×10^{7}

DPM/mg; 75% ^{3}H-PE, 8.5 x 10^6 DPM/mg; 62% ^{3}H-PE, 6.03 x 10^6 DPM/mg.

Pillbugs (*Armadilidium vulgare*) were collected locally. The pillbug assays were conducted in plastic culture dishes containing sea sand (Fisher Scientific Company, Fair Lawn, NJ), moistened with distilled water. All containers were incubated in the dark at 20 °C. Each sample dish contained 3 pillbugs along with one starch/^{3}H-PE disk (50 mm^2) cut into 3 pieces of equal size. Treatments included (1) 100% ^{3}H-PE, (2) 75% ^{3}H-PE/15% oxidized PE/10% starch, (3) 62% ^{3}H-PE/13% oxidized PE/25% starch, and (4) a control sample with no polyethylene disk. Observations, taken on a daily basis, included degree of disk consumption and pillbug survival. Consumption was evaluated using three categories: (1) no consumption, (2) less than 50% consumption, and (3) greater than 50% consumption over the 7-d exposures.

At the conclusion of the experiment, compartments (sand, frass, bodies) were analyzed to determine the location(s) of the radioactivity and the polarity of the degradation products. All samples were extracted by the procedure of Bligh and Dyer (1959) with modifications for different samples. Samples were homogenized with 2:1 (vol:vol) chloroform:methanol for 2 hours. The extract was split into two phases (aqueous and organic) by adding $CHCl_3$ and H_2O, and degradation products were allowed to partition between the phases for 24 h. Radioactivity in each phase was determined using an LKB Wallac (LKB Instruments, Rockville, MD) liquid scintillation spectrometer. Sample quenching was determined using a quench curve consisting of ^{3}H standards. The residue remaining after homogenation and extraction of the pillbugs was dried under N_2, weighed, and combusted using a Packard Model 306 sample oxidizer (Packard Instruments, Downers Grove, IL) and the radioactivity determined as described above. Extracted sand was also combusted to determine the presence of particulate disk pieces and/or frass not removed by extraction. The efficiency of the combustion procedure was determined using ^{3}H-Spec-Chec (Packard Instruments, Downers Grove, IL).

As part of the experimental controls, small portions of the three starch/PE samples (100% ^{3}H-PE, 75% ^{3}H-PE, and 62% ^{3}H-PE) were extracted and partitioned using the procedure described above. Radioactivity in the two phases (aqueous and organic) was determined to provide an indication of the extractability and solubility of the parent (undegraded) material.

RESULTS AND DISCUSSION

Extraction and partitioning controls for the parent (undegraded) plastic samples are shown in Table 1. Radioactivity in the aqueous- and organic-soluble fractions was related to the initial activity of the sample type. Namely, the 100% ^{3}H-PE sample, which had the highest initial

Table 1. Distribution of radioactivity (^3H) expressed as disintegrations per minute (DPM) in various aqueous- and organic-soluble fractions after extraction and partitioning of ^3H-labelled PE disks containing 0-25% starch.

	Radioactivity (DPM)		
Sample Type	Initial[A]	Aqueous-Soluble Fraction	Organic-Soluble Fraction
Control[B]	0	35	57
100% ^3H-PE	7.90×10^6	73	10,476
75% ^3H-PE[C]	4.42×10^6	42	2,833
62% ^3H-PE[D]	2.05×10^6	35	270

[A]Amount of radioactivity initially contained in the disk.
[B]Control contained no sample disk and is an indication of background radioactivity.
[C]Disk composed of 75% ^3H-PE, 15% oxidized PE, and 10% starch by weight.
[D]Disk composed of 62% ^3H-PE, 13% oxidized PE, and 25% starch by weight.

activity (7.90 x 10^6 DPMs), had the most radioactivity in the organic and aqueous fractions. As no cleanup was carried out on the ^{3}H-PE produced from the reduction reaction described above, the samples may have contained impurities (toluene) which acquired ^{3}H atoms from exchange with the ^{3}H-poly-1,3 butadiene during storage before the reduction. Nonetheless, radioactivity was minimal compared with the initial radioactivity, especially in the aqueous-soluble fraction. Importantly, the radioactivity in the fractions decreased to background levels during a subsequent second extraction (data not shown).

Survival of the pillbugs during the 7-d experiment was 100% in control and treatment samples. After 2 days, the disk pieces in the 75% and 62% ^{3}H-PE treatments were completely consumed based on visible examination, whereas the disk pieces in the 100% ^{3}H-PE treatment were still present at day 7, and based on visible examination, did not appear to be consumed. This was further illustrated by the fact that the weight of the 100% ^{3}H-PE disk pieces on day 7 was 98% of the day-0 weight.

The distribution of radioactivity in the various compartments analyzed is shown in Table 2. Surprisingly, even the 100% ^{3}H-PE disk samples appear to have been metabolized to some degree based on the presence of aqueous- and organic-soluble radioactivity above background (control) levels in sand and frass from the sample containers. In addition, organic-soluble radioactivity was also detected in the pillbug bodies for these treatments. There was no correlation between the size of the pillbug and the total amount of radioactivity contained in the body.

Radioactivity in the aqueous-soluble fraction of the sand and frass compartments increased with higher starch content of the disk. Organic-soluble radioactivity was also detected in the frass and sand compartments, as well as the pillbug bodies.

Although the 75% and 62% ^{3}H-PE films were completely consumed, mass balances based on radioactivity for the treatments were quite low. The exception to this is the 100% ^{3}H-PE treatment. As 3 disk pieces accounting for 98% of the initial weight were removed on day 7, the mass balance for this treatment was nearly 100%. However, for the other treatments, mass balances were substantially lower (< 10%). It is possible that most of the radioactivity escaped from the system as ammonia or other volatile organic compounds. Terrestrial isopods are known to excrete ammonia as a waste product (Sutton and Holdich, 1984).

These studies were conducted to elucidate the potential role of soil macroinvertebrates in degrading starch/polyethylene biodegradable plastics and to determine the solubility of the degradation products. Previous bioassays with a variety of macroinvertebrates indicated that starch-containing polyethylene films were consumed to various degrees

Table 2. Distribution of radioactivity (^3H) expressed as disintegrations per minute (DPM) in various compartments after 7-d exposure to ^3H-labeled PE/starch disks. Samples were extracted and partitioned between an aqueous fraction and an organic fraction. Solid residue remaining after partitioning was oxidized and the ^3H was trapped as water vapor.

Treatment	Radioactivity (DPM)								
	Sand			Frass		Body[A]			
	Aqueous	Organic	Residue	Aqueous	Organic	Aqueous	Organic	Residue	Weight[B]
Control	37	66	3,675	36	75	32	62	238	5.7
						36	77	225	30.7
						34	72	230	14.0
100% ^3H-PE[C]	423	1,172	61,992	68	164	40	157	254	17.7
						43	3,071	1,134	6.6
						39	253	253	7.2
75% ^3H-PE[D]	1,067	883	50,535	618	18,310	40	2,297	2,608	80.7
						62	9,608	6,191	12.5
						44	248	277	10.6
62% ^3H-PE[D]	2,334	1,118	37,258	882	6,540	76	3,147	5,033	49.8
						36	111	193	5.2
						42	210	323	12.2

[A]Each pillbug was analyzed individually.
[B]Dry weight of pillbug residue in milligrams.
[C]3 disk pieces totaling 4.11 mg (98% of the initial disk weight at time 0) were removed from the sand on day 7.
[D]No disks were remaining at the conclusion of the experiment.

depending on starch content (Wool, 1988). We observed that the primary degradation of starch/PE films could be carried out by a variety of soil macroinvertebrates including crickets and isopods (Tsao et al., 1993). Although the studies did not identify the metabolic products produced by the degradation, physical deterioration, which is one of the crucial factors for complete degradation of the plastic film, did occur. The results reported herein indicate that quantitatively, plastic disks containing 10 and 25% starch are completely consumed by terrestrial isopods. More importantly, aqueous soluble degradation products of the PE are formed during consumption. In other words, the PE molecule does not pass through the isopods molecularly unchanged.

These results have important implications for determining the fate of novel plastic formulations in compost environments and as discarded polymers. Certainly variables inherent in seasonal and geographic variation will be important in determing the extent to which soil isopods influence the fate of degradable plastics.

The use of ^{3}H-PE could be particularly productive when blended with ^{14}C-starch. Identification of the component of the plastic being degraded would be easily detected and elucidated using these dual-labeled films. Studies such as these with macroinvertebrates, coupled with studies on microbial degradation (Burgess-Cassler et al., 1991; Lee et al., 1991; Pometto et al., 1992) help provide the type of information needed to assess the environmental fate of biodegradable starch/PE plastics.

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