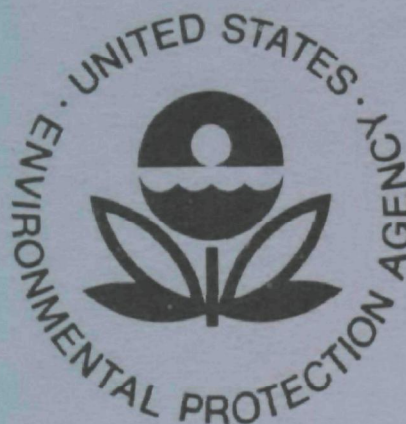


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DEGRADATION MECHANISMS: CONTROLLING THE BIOACCUMULATION OF HAZARDOUS MATERIALS



**National Environmental Research Center
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U.S. Environmental Protection Agency
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DEGRADATION MECHANISMS:
CONTROLLING THE BIOACCUMULATION
OF HAZARDOUS MATERIALS

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FOREWORD

Man and his environment must be protected from the adverse effects of pesticides, radiation, noise and other forms of pollution, and the unwise management of solid waste. Efforts to protect the environment require a focus that recognizes the interplay between the components of our physical environment--air, water, and land. The National Environmental Research Centers provide this multidisciplinary focus through programs engaged in

- studies on the effects of environmental contaminants on man and the biosphere, and
- a search for ways to prevent contamination and to recycle valuable resources.

As a part of these activities, the study described here documents the existence of natural hazardous waste transformation processes in the environment and the need for implementation of control technology.

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ABSTRACT

Many hazardous materials are manufactured for specific industrial and agricultural applications. These materials often are applied for beneficial uses, and are accidentally spilled, or released through waste streams into the environment. This study documented the existence in the environment of biological, chemical, and physical transformation processes for hazardous materials. Studies determined that the slow rate of transformation of these hazardous materials results in the persistence and bioaccumulation of certain potentially harmful residues in living systems. A scheme to minimize the release of unwanted hazardous chemicals into the environment is described.

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DEGRADATION MECHANISMS: CONTROLLING THE BIOACCUMULATION OF HAZARDOUS MATERIALS

INTRODUCTION

In recent years, the release of synthetic organic chemicals into the environment has greatly increased. Although many of these materials are decomposed naturally by chemical, physical, and biodegradative processes, a surprisingly large number of the synthetic compounds (or products formed from them) are destroyed at rates too slow to prevent undesirable accumulations in the environment. Because of the threat to public health, these compounds are attracting increasing attention. One example of such compounds are the persistent insecticides that are widely used in the temperate zone for food production and in the tropics for the control of insects bearing human pathogens, and herbicides that remain in the soil for years are important in the agricultural industry.

Persistence or nonbiodegradability is frequently a desirable characteristic of a synthetic compound. Because of their low cost, low acute toxicity to man, and long period of effect, certain persistent insecticides remain the best means to control the insect vector of malaria in developing countries. By suppressing weeds for one or several seasons, persistent herbicides cost less for weed control in agricultural areas and along highways and railroads. Similarly, resistance of fabrics and packaging materials to biodeterioration during the period of their use is a desirable feature.

On the other hand, resistance to natural degradation is quite often an undesirable property. For example, components of industrial waste effluents introduced into waterways, if not decomposed by the aquatic microflora, can harm wildlife, reduce the quality of drinking water, and result in the bioaccumulation of hazardous chemicals. Toxic accumulations of persistent insecticides can enter living systems by means of: absorption into earthworm tissues, then into birds feeding on the worms; soil erosion into adjacent bodies of water, then into fish or other aquatic life; and absorption into the roots of growing crops, then into the livestock or humans who eat the crops. Phytotoxicity has been reported in many areas where a persistent herbicide applied to a resistant crop was not degraded by the time a succeeding but sensitive crop was sown in the field, and farmers have sometimes incurred appreciable financial losses from residual toxicity of herbicides used in a crop rotation.¹

Resistance to degradation can also result in the presence of DDT, aldrin, polychlorinated biphenyls, o-chloronitrobenzene, and many other compounds in rivers and drinking water. However, the possible hazard to man's health of a long-term exposure to low concentrations of most nonbiodegradable pollutants is unknown. Furthermore, in spite of rapid developments in toxicology, previously unrecognized or even untested effects on human health could become evident several years from now as the result of the introduction of a synthetic organic chemical into the environment. If the chemical compound was susceptible to natural degradation, cessation of human use would result in the disappearance of the newly recognized toxicant from ecosystems. By contrast, if the compound was nonbiodegradable, its harmful effects would continue for months, years, or decades, because of the absence of an effective means to rapidly eliminate the offending chemical from natural ecosystems. Thus, the widespread dissemination of nonbiodegradable or persistent chemicals is an inherent risk, since detection and control of their toxic effects is contingent and unreliable.

The purpose of the report is to delineate the mechanisms that transform hazardous materials into toxically active and inactive compounds and to describe treatment a scheme that minimizes the release of unwanted materials into the environment.

Biodegradation

Studies on pesticide metabolism in insects have provided considerable information on the interactions between the toxic chemicals and the mechanisms of species resistance. The pathways of pesticide metabolism were determined primarily with in vivo degradation, and metabolities were recovered mostly as secondary water soluble products. A more recent study,² has involved the use of individual insect tissues, cellular preparation, or both in an effort to characterize primary metabolites. Results clearly established the important role of hydrolysis, oxidation, reduction, dealkylation, desulfuration, and dehalogenation in the biotransformation of organochlorine, organophosphorus, and carbamate insecticides in insects. Some of the basic enzymatic reactions in pesticide metabolism are shown in Table 1.³ Mechanisms for the metabolic transformation of pesticides containing aromatic structures are given in Figure 1. In principle, all enzyme-catalyzed reactions are thermodynamically reversible; in some instances, however, the equilibrium is so far in one direction that it precludes both the reverse reaction or the resynthesis of the parent compound. The function of the appropriate enzyme is to catalyze whenever feasible, the attainment of equilibrium in either direction.

Type Reactions	Enzyme Group
1. Hydrolysis-condensation or replacement	
$\text{RCO}-\text{NHR}' + \text{H}_2\text{O} \rightleftharpoons \text{RCOOH} + \text{R}'\text{NH}_2$ $\text{RCO}-\text{NHR}' + \text{R}''\text{NH}_2 \rightleftharpoons \text{RCO}-\text{NHR}'' + \text{R}'\text{NH}_2$	Proteinases, peptidases, and amidases
$\text{RCO}-\text{OR}' + \text{H}_2\text{O} \rightleftharpoons \text{RCOOH} + \text{R}'\text{OH}$ $\text{RCO}-\text{OR}' + \text{R}''\text{OH} \rightleftharpoons \text{RCO}-\text{OR}'' + \text{R}'\text{OH}$	Esterases
$\text{RCO}-\text{SR}' + \text{H}_2\text{O} \rightleftharpoons \text{RCOOH} + \text{R}'\text{SH}$ $\text{RCO}-\text{SR}' + \text{R}''\text{H} \rightleftharpoons \text{RCO}-\text{R}'' + \text{R}'\text{SH}$	Thiol esterases
$\text{R}-\text{PO}_3\text{H}_2 + \text{H}_2\text{O} \rightleftharpoons \text{RH} + \text{H}_3\text{PO}_4$ $\text{R}-\text{PO}_3\text{H}_2 + \text{R}'\text{OH} \rightleftharpoons \text{RH} + \text{R}'\text{O}-\text{PO}_3\text{H}_2$ $\text{R}-\text{PO}_3\text{H}_2 + \text{R}'\text{NH}_2 \rightleftharpoons \text{RH} + \text{R}'\text{NH}-\text{PO}_3\text{H}_2$	Phosphatases and transphosphorylases
$\text{R}-\text{CH}-\text{OR}' + \text{H}_2\text{O} \rightleftharpoons \text{RH} + \text{HO}-\text{CH}-\text{OR}'$	Glycosidases*
$\text{R}-\text{CH}-\text{OR}' + \text{R}''\text{H} \rightleftharpoons \text{RH} + \text{R}''-\text{CH}-\text{OR}'$	Transglycosidases*
2. Phosphorolysis-condensation	
$\text{R}-\text{CH}-\text{OR}' + \text{H}_3\text{PO}_4 \rightleftharpoons \text{RH} + \text{H}_2\text{O}_3\text{PO}-\text{CH}-\text{OR}'$	Phosphorylases*
3. Cleavage or formation of C—C linkages	
$\text{RCOOH} \rightleftharpoons \text{RH} + \text{CO}_2$	Decarboxylases
$\begin{array}{c} \text{H} \quad \text{H} \\ \text{HO}-\text{C}-\text{C}-\text{OH} \\ \text{R} \quad \text{R}' \end{array} \rightleftharpoons \text{RCH}_2\text{OH} + \text{R}'\text{CHO}$	Aldolases
4. Hydration-dehydration and related processes	
$\begin{array}{c} \text{H} \quad \text{H} \\ \text{R}_2\text{C}-\text{C}-\text{OH} \\ \text{R} \end{array} \rightleftharpoons \begin{array}{c} \text{H} \\ \text{R}_2\text{C}=\text{C} \\ \text{R} \end{array} + \text{H}_2\text{O}$	Hydrases and related enzymes (elements of H ₂ O or NH ₃ may be replaced by those of H ₂ S)
$\begin{array}{c} \text{H} \quad \text{H} \\ \text{R}_2\text{C}-\text{C}-\text{NH}_2 \\ \text{R} \end{array} \rightleftharpoons \begin{array}{c} \text{H} \\ \text{R}_2\text{C}=\text{C} \\ \text{R} \end{array} + \text{NH}_3$	
5. Oxidation-reduction	
$\text{AH}_2 + \text{B} \rightleftharpoons \text{A} + \text{BH}_2$	Dehydrogenases
$2\text{Fe}^{2+} + \frac{1}{2}\text{O}_2 + 2\text{H}^+ \rightarrow 2\text{Fe}^{3+} + \text{H}_2\text{O}$	Oxidases
$\text{AH}_2 + \text{O}_2 \rightarrow \text{A} + \text{H}_2\text{O}_2$	
$\text{AH}_2 + \text{H}_2\text{O}_2 \rightarrow \text{A} + 2\text{H}_2\text{O}$	Peroxidases and catalases
$2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$	

*The type formula $\text{R}-\text{CH}-\text{OR}'$ denotes a glycoside

Table 1 Basic enzymatic reactions in pesticide metabolism.

Induced Formation of Bacterial Enzymes. Many microorganisms are able to "adapt" to the utilization of one of a variety of substances added to cultural medium by forming an enzyme system that is not evident when the organism is grown in the absence of the added substance. This phenomenon is termed "enzyme induction," and the substance (i.e., pesticide, etc.) that initiated the response is the enzyme-inducing agent. For example, the enzyme B-galactosidase appears in *Escherichia coli* when the organism is grown in the presence of B-galactosides such as lactose (a substrate of the enzyme) or methyl-B-D-thiogalactoside (which is not hydrolyzed by the enzyme).⁴ Thus, an inducer need not be the substrate of the enzyme whose formation it invokes; yet, it appears that the inducer serves as a template (perhaps in stimulating messenger RNA) which plays a role in the synthesis of intracellular enzymes. The pesticides also participate in some manner that elicits the response as an enzyme inducer.

Pesticide Metabolism. Pesticides that are transformed by biological systems include parathion, methyl parathion, DDT, dieldrin, 2,4-D, MCPA, silvex, fenac, dalapon, atriazine, and DCPA.⁵ Pesticides are metabolized both by the target organisms at which they are directed and by man and other nontarget species. The herbicide 3,4-dichloropropionanilide (DCPA) is an example of a compound whose metabolism results in a hazardously persistent residue.⁶

Bartha and Promer determined that DCPA is metabolized by a number of soil microorganisms; they suggested that the labile aliphatic side chain of the molecule is oxidized in part to carbon dioxide and that the aromatic moiety is liberated as a toxic residue that depressed soil respiration. By using fractionation procedures on soil samples containing DCPA, they identified two decomposition products--the metabolites 3,4-dichloroaniline (DCA) and 3,3,4,4-tetrachloroazobenzene (TCAB). The scheme of metabolic transformation of 3,4-dichloropropionanilide in soil is illustrated in Figure 2.

Acylamidase of microbial origin in the soil catalyzes the cleavage of the herbicide DCPA into DCA and propionic acid. The propionic acid is utilized as a source of carbon and energy by soil microorganisms. The condensation that produces TCAB from DCA may be a direct oxidative condensation of two molecules of DCA; or DCA may be first transformed in part to 3,4-dichloro-nitrosobenzene, after which a spontaneous condensation occurs between one molecule of the aniline compound and one molecule of nitroso. Because of these condensation reactions, DCPA accumulates



in the soil as TCAB, an ozo compound; in the Barth and Promer study,⁶ 46% of the DCPA was recovered as TCAB 30 days after application of the herbicide. Further study on the metabolites from decomposition of this compound and all other pesticides is greatly needed.

Foreign chemicals, when introduced into higher living organisms such as man, undergo metabolic transformation. Transformations of the parent molecules are enzymatically induced by naturally occurring and synthetic substances. Williams⁷ divided the biotransformation mechanisms of foreign chemicals into two major types: the nonsynthetic reactions involving reduction and hydrolysis, and the synthetic reactions involving biosynthesis of a product from the chemical and from an endogenous metabolite. The biotransformation reactions are especially important in insects, since most of the widely used pesticides are not effectively toxic until they are metabolized in the host. Metabolic conversion of a chemical frequently results in the formation of products that are more toxic than the original compounds.⁸

Microsomal drug enzymes in the liver cells protect humans and animals from the effects of foreign chemicals by catalyzing a variety of biotransformation reactions--including hydroxylation, dealkylation, deamination, alkyl side chain oxidation, hydrolysis, and reduction. Moreover, the total quantity of microsomal enzymes is increased significantly when insecticides such as chlordane, DDT, hexachlorocyclohexane, dieldrin, aldrin, and heptochlor are administered repeatedly to animals. Yet, the final role of microsomal-metabolizing enzymes in humans and animals after exposure to toxic chemicals remains virtually unknown. Because of the uncertainty of the effects of these enzymes, technology should be developed and implemented to safeguard public health from unnecessary exposure to these enzymes-inducing materials.

Photodegradation

Maugh⁹ reported that DDT vapors can be converted into polychlorinated biphenyl's (PBC's) by irradiation with ultraviolet light of the same wavelengths present in sunlight. This conversion is a natural mechanism that produces a transformation product as toxic as or more toxic than the parent chemical. In this natural degradation process, the absorption (facilitated by a photosensitizer) of irradiated solar energy into an insecticide, pesticide, or herbicide chemical increases molecular energy. Persisting for a relatively long period of time at this high energy level, these activated molecules release their absorbed energy, thereby effecting

the photolysis of the pesticides and of their residues. With free access to air and under illumination from sunlight or ultraviolet light, hydroxyl groups replace halogens in a number of pesticides in aqueous solution. The irradiation in water of such herbicidal compounds such as chlorobenzoic acids and amiben (3-amino-2, 5-dichlorobenzoic acid) with light of a wavelength directly absorbed by the molecules leads to replacement of chlorine by both hydrogen and hydroxyl. Picloram (4-amino-3,5,6-trichloro-picolinic acid) is degraded by light under a variety of conditions. Photolysis of picloram takes place in aqueous solution and in the solid phase. In a study by Plimmer,¹⁰ 1 mg of picloram applied to the surface of a petric dish and irradiated with ultraviolet light was degraded 60 percent after 48 hours and 90 percent after 1 week.

The compound 3,4-dichloroaniline (DCA), whose transformation in the soils to tetrachloroazobenzene (TCAB) by microbial was discussed earlier, is also transformed by photolysis. With the aid of the photosensitizer benzophenone, Plimmer and Kearny¹¹ photolyzed DCA in benzene solution to TCAB; and, even though preliminary, their results clearly determined that the photo-products of DCA were chemically identical to TCAB. Plimmer and Kearney also demonstrated that 3,4-DCA is photolyzed in ultraviolet light with riboflavin as the photosensitizer.

The widely used S-triazine group of herbicides has not yet been thoroughly studied photochemically. Many reviews on pesticides describe their inactivation by ultraviolet energy and by infrared irradiation. It has not been clearly resolved whether loss of pesticidal potency is due to volatilization or photodecomposition.¹² It is also assumed that microbial and photodegradation reactions play a major role in reducing the presence of persistent toxic residues in the environment.

Bioaccumulation

In 1969 Heyndrick and Maes¹³ compared the concentrations of organic chlorinated pesticides in the lipid fraction of mother's milk with the accumulation of these pesticides in baby fat. Twenty samples of human milk were analyzed quantitatively for pesticide residues. The results revealed that babies having human milk as a sole food source are exposed to a higher dietary insecticide concentration than the general population.

Presented in the Journal of the American Medical Association,¹⁴ a discussion of organophosphorus pesticides emphasized their mechanism(s) of toxic action in the human body. Under certain

circumstances, these relatively nontoxic pesticides (chemically very similar to nerve gas) can be converted in the body to extremely deadly poisons. In insects, inhibition of acetylcholine esterase results in the accumulation of a lethal concentration of acetylcholine. The same inhibition of acetylcholine esterase takes place in mammals, but here rapid metabolism of the toxic substance minimizes the effect. As long as the insecticide is decomposed more rapidly than acetylcholine is formed, there is no danger to health.

Accumulation of chemicals in a living cell requires that sufficient amounts of the chemicals approach the cell membrane and that the cell membrane allows their entry into the cell. If removed from the environment of the foreign chemical in time, the organism or cell usually avoids the toxic effect of the chemical. Elimination rates of chemicals from organisms depend upon the nature of the chemical and the mechanism used to remove the chemical from the organism.

The elimination rates of chlorinated hydrocarbons were determined in a study in New Zealand.¹⁵ Heifers and lambs were dosed daily for 16 weeks with a mixture of DDT and dieldrin and were then maintained for 32 weeks without dosing. More dieldrin than DDT accumulated in the fat of both animals, and lambs stored more dieldrin than heifers. Dieldrin and DDT were present in both animals 32 weeks after the final dose.

Heavy metals are also accumulated by living tissues. One of the heavy metals, mercury, occurs in the compound phenyl mercury acetate, a major agricultural chemical. In 1968 H. Tokutomi¹⁶ conducted a follow-up study on mercury poisoning cases resulting from use of this compound. Extensive neurological disorders persisted with little or no improvements 10 years after the initial poisoning. Not surprisingly, Tokutomi reported there are, as one would suspect, many cases of heavy metal poisoning of humans in developing countries. For example, the Ministry of Labor in El Salvador recorded 2028 cases of poisoning (including 30 fatalities) of humans in 1972. In addition, this data showed that the problem of the persistence of pesticides residues such as DDT and dieldrin in grazing pastures, in cottonseed oil, and in corn fed to cattle posed a severe threat to domestic health. Because of the potentially harmful (though frequently unknown) effects of accumulated agricultural chemicals, technology that prevents the release of these chemicals into the ecosystem should be developed and implemented.

Technology Alternatives

To reduce the impact of toxic and hazardous materials (whether in liquid, semi-liquid, or solid form) on the environment, several techniques have been developed and new promising technologies are continuously being developed. Though in the past these unwanted waste materials have been disposed by the quickest, easiest, and most economical means, a recent increase in the supply of agricultural chemicals and (more importantly) an increase in concern for the environment places new emphasis on the implementation of treatment processes prior to disposal. An acceptable approach to concentrate, process, and reintroduce (when applicable) hazardous materials to the market is schematically illustrated in Figure 3.

The physical state, chemical concentration, and recovery potential of the waste stream determines the most economically feasible processing route. Some waste streams could offer limited resource recovery potential, require little or no concentration, and could be disposed of directly. Semi-liquids and liquids could be recovered for reuse or disposed of directly. However, in some cases, a concentration and/or a detoxification step is needed to safely recover the hazardous materials from the waste stream.

The concentration process includes techniques employing reverse osmosis, ion exchange, activated carbon adsorption, and impoundment. The nature of the influent and the extent of concentration required would determine the best concentration procedure. Most of these processes have been developed, and recent advances¹⁷ make them attractive for implementation in the control of hazardous waste.

The detoxification process is necessary to treat those materials that no longer require the toxic element or compound. Techniques that could be employed to detoxify the waste materials include chemical oxidation/reduction, catalytic, and substitution/transformation processes. A product assessment would be necessary to ensure that the detoxification process has been completed and that nothing harmful from the original hazardous waste stream remains in the recovered products.

If implemented, any of these detoxification processes would probably generate waste that requires disposal. Disposal methods that could be used include ocean and deep well disposal, sanitary landfills, encapsulation, and incineration. However, ocean and deep well disposal are becoming unacceptable because of the inability to control the possibly harmful effects of these waste materials once they are placed in the sea or deep well environ-

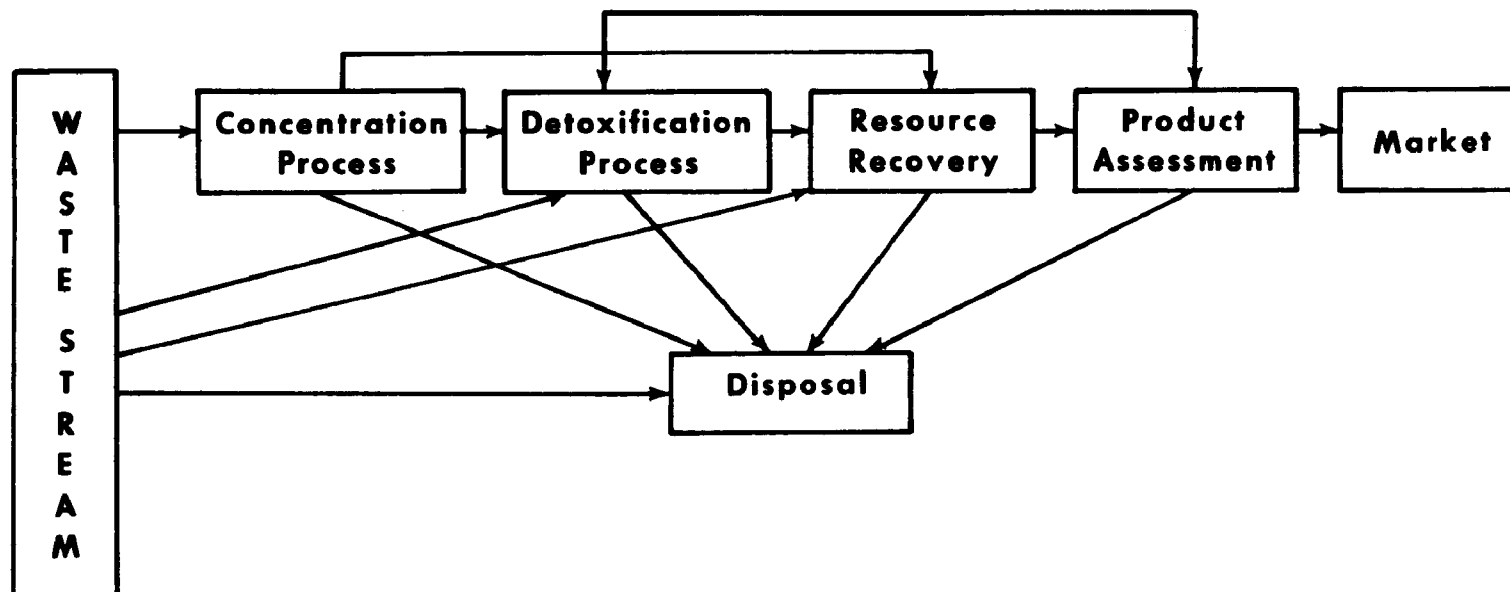


Figure 3 Scheme for processing hazardous waste for recovery and/or disposal

ments; and incineration produces gases and residues that must be controlled.

Specialized techniques for detoxification include wet oxidation and chlorinolysis. A chemical analysis of the waste stream and potential market of the end products usually dictates the feasibility of one technique over another. End products could be disposed of directly or resubmitted into the market recovery and reuse stream. This resource recovery step in the above scheme could involve additional concentration, identification of potential useful products, and purification processes.

Before the return of material that once was toxic and hazardous to the recovery and reuse stream, extensive short- and long-term toxicological evaluation should be performed. Exceptions to this are those products recovered for use as originally intended (i.e., pesticides, organics, etc.).

SUMMARY

The mechanisms for degradation of hazardous materials in nature are chemical, biological, and photodegradation processes. Many hazardous materials are destroyed by these naturally occurring processes but at rates too slow to prevent significant and unwanted accumulation in living systems. Because natural transformation of certain hazardous materials often leads to the synthesis of a compound more toxic than the parent chemical compound, nonbio-degradable structures can result in detectable and potentially harmful bioaccumulation in living systems. Therefore, safe technologies for resource recovery and waste disposal should be developed and implemented to minimize the undesirable release of hazardous materials into the environment.

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16. ABSTRACT <p>Numerous toxic and hazardous compounds are being generated for commercial, industrial and agricultural uses. Most of these materials are eventually released into the environment. This study documented the existence of biological, chemical and physical transformation processes of hazardous wastes in the environment. It was determined that the rates of transformation of these hazardous materials, when applied and/or discharged to the environment, are slow. Case studies confirmed that persistency of certain hazardous materials results in the bioaccumulation of residues in living systems. A lack of knowledge of the fate and clinical manifestation of persistent residues in living systems dictates the need for implementation of the described scheme of control technology.</p>		
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