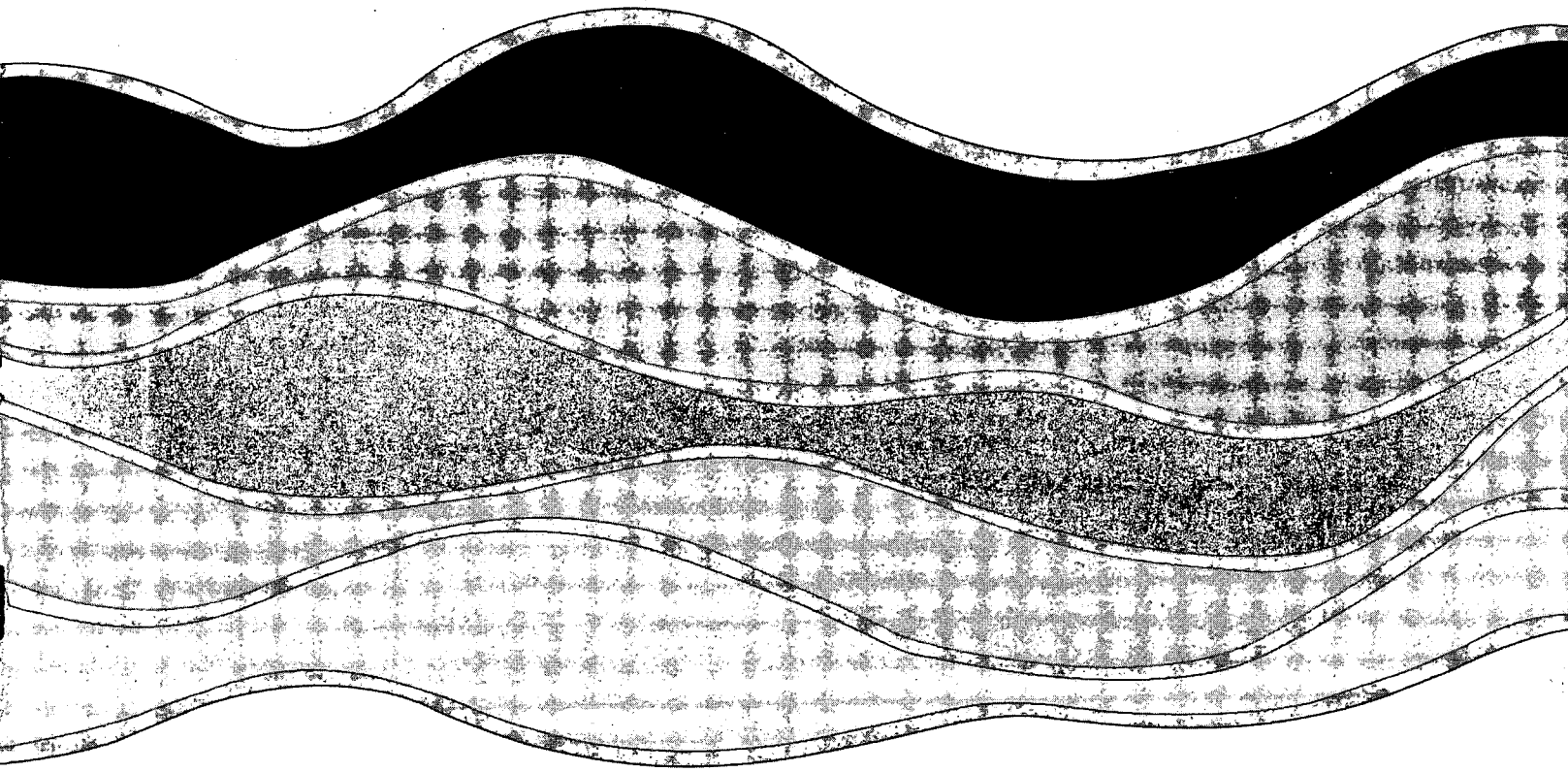
NWRI Cont
96-175

NATIONAL WATER RESEARCH INSTITUTE

INSTITUT NATIONAL de RECHERCHE sur les EAUX



MICROBIAL ADSORPTION OF CYANAZINE AND METOLACHLOR

D. Liu, J. Maguire, L. Lau, G. Pacepavicius, H. Okamura and I. Aoyama

NWRI Contribution No. 96-175

Microbial Adsorption of Cyanazine and Metolachlor

D. Liu[1], R.J. Maguire[1], Y.L. Lau[1], G.J. Pacepavicius[1],
H. Okamura[2] and I. Aoyama[2]

[1]National Water Research Institute
Environment Canada
Burlington, Ontario, Canada L7R 4A6

[2]Research Institute for Bioresources
Okayama University, Kurashiki, Japan 710

Contribution No. 96-175

MANAGEMENT PERSPECTIVE

The accurate assessment of a pesticide's persistence and fate(in the laboratory or *in situ*) is not always easy and can be complicated by incomplete recovery of the pesticide from the test medium or environmental samples. Until recently, the issue of extractability (recovery) has received little attention in the pesticide literature. Although many factors can affect this recovery, the tendency for pesticides to complex with soil constituents and biomass to form nonextractable residues is especially significant. This may have a far-reaching impact on the issue of pesticide management, due to the potential underestimation of the levels of total pesticide residues in the environment.

A laboratory experiment was performed to study the role of microorganisms in producing these bound residues by anaerobically incubating cyanazine (2-[[4-chloro-6-(ethylamino)-1,3,5,-triazin-2-yl]-amino]-2-methylpropanenitrile) and metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide] in a culture medium that had been inoculated with a PAH-degrading bacterial culture. This study provided the first direct evidence that bacterial biomass rather than metabolism was mainly involved in the formation of bound residues with cyanazine, based on the GC analyses of extracts from the degradation, adsorption, and abiotic controls for the parent herbicide and its possible metabolites. However, anaerobic bacteria appeared to be incapable of forming bound residues with metolachlor. The common phenomenon of age-dependent extractability for bound residues was also observed with cyanazine. The apparent irreversible adsorption of cyanazine by bacterial biomass was not affected by the presence of metolachlor in the test system, thus implying that bacteria may adsorb pesticides selectively with preference for a certain chemical structures over others.

Cyanazine and metolachlor are important selective herbicides used for the control of several annual grassy weeds and certain broad-leaf weeds in fields of corn, soybeans, and triazine-resistant canola. They are two of the 10 most heavily used agricultural herbicides in Ontario. An estimated 4.2×10^3 metric tons of agricultural herbicides (active ingredients) of all types were used in Ontario in 1993. More than 46% of this total was cyanazine and metolachlor. Both herbicides have been detected in river waters and well waters in Ontario. In order to make an assessment of the hazards of cyanazine and metolachlor use to aquatic ecosystems, more information is required on their persistence and fate. There is virtually no information in the open literature on the interactions between bacterial biomass and cyanazine. This work fills research needs identified by the Ecosystem Interpretation Branch in the development of Canadian Water Quality Guidelines for cyanazine, and by the U.S. Environmental Protection Agency in the course of pesticide re-registration.

SOMMAIRE À L'INTENTION DE LA DIRECTION

L'évaluation exacte de la persistance et du devenir d'un pesticide (au laboratoire ou *in situ*) n'est pas toujours facile et peut être compliquée du fait de la récupération incomplète du pesticide du milieu d'essai ou d'échantillons prélevés dans l'environnement. Jusqu'à récemment, la question de l'extractibilité (récupération) a reçu peu d'attention dans la littérature sur les pesticides. Bien que de nombreux facteurs puissent influencer cette récupération, la tendance des pesticides de former des complexes avec les constituants du sol et la biomasse, ce qui donne des résidus non extractibles, est particulièrement importante. Ce problème peut avoir des répercussions considérables sur la question de la gestion des pesticides, à cause de la sous-estimation éventuelle des concentrations des résidus totaux de pesticides dans l'environnement.

On a effectué une expérience en laboratoire pour étudier le rôle des microorganismes dans la production de ces résidus liés. Pour ce faire, on a incubé de la cyanazine (2-[[4-chloro-6-(éthylamino)-1,3,5-triazin-2-yl]-amino]-2-éthylpropanenitrile) et du métolachlore (2-chloro-N-(2-éthyl-6-méthylphényl)-N-(2-méthoxy-1-méthyléthyl) acétamide) en anaérobiose dans un milieu de culture ayant été inoculé avec une culture bactérienne qui dégrade les HAP. Cette étude a fourni la première preuve directe que la biomasse bactérienne, plutôt que le métabolisme, est principalement responsable de la formation de résidus liés avec la cyanazine, à partir des analyses par CG d'extraits provenant de la dégradation et de l'adsorption, et de contrôles abiotiques pour l'herbicide de départ et ses métabolites éventuels. Toutefois, les bactéries anaérobies semblaient incapables de former des résidus liés avec le métolachlore. Le phénomène courant de l'extractibilité selon l'âge des résidus liés a également été observé dans le cas de la cyanazine. L'adsorption apparemment irréversible de la cyanazine par la biomasse bactérienne n'était pas influencée par la présence de métolachlore dans le système d'essai, ce qui implique que les bactéries peuvent adsorber les pesticides de manière sélective et se lier de préférence à certaines substances chimiques plutôt qu'à d'autres.

La cyanazine et le métolachlore sont des herbicides sélectifs importants dans la lutte contre plusieurs mauvaises herbes graminées annuelles et certaines dicotylédones dans les champs de maïs, de soja et de canola résistant à la triazine. Il s'agit de deux des dix herbicides les plus utilisés en agriculture en Ontario. On a estimé à $4{,}2 \times 10^3$ tonnes métriques la quantité d'herbicides agricoles (ingrédients actifs) de tous types qui a été utilisée en Ontario en 1993. La cyanazine et le métolachlore représentaient plus de 46 % de cette quantité. Les deux herbicides ont été détectés dans des cours d'eau et des puits en Ontario. Il faut obtenir plus de données sur la persistance et le devenir de la cyanazine et du métolachlore pour évaluer les dangers que représente leur utilisation pour les écosystèmes aquatiques. Il n'y a pratiquement pas d'information dans les documents publiés sur les interactions entre la biomasse bactérienne et la cyanazine. Le présent travail comble les besoins de recherche déterminés par la Direction de l'interprétation des écosystèmes dans l'élaboration de Recommandations pour la qualité des eaux au Canada pour la cyanazine, et par la U.S. Environmental Protection Agency dans le cadre de la ré-homologation des pesticides.

ABSTRACT

Many pesticides can form bound residues (residues not recovered by standard Soxhlet extraction techniques) after application, thus enhancing their environmental persistence. A laboratory experiment was performed to study the role of microorganisms in producing these bound residues by anaerobically incubating cyanazine (2-[[4-chloro-6-(ethylamino)-1,3,5,-triazin-2-yl]-amino]-2-methylpropanenitrile) and metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide] in a culture medium that had been inoculated with a PAH-degrading bacterial culture. Based on the GC analyses of extracts from the degradation, adsorption, and abiotic controls for the parent herbicide and its possible metabolites, this study provided the first direct evidence that bacterial biomass, rather than metabolism, was mainly involved in the formation of bound residues with cyanazine. However, anaerobic bacteria appeared to be incapable of forming bound residues with metolachlor. The common phenomenon of age-dependent extractability for bound residues was also observed with cyanazine. The apparently irreversible adsorption of cyanazine by bacterial biomass was not affected by the presence of metolachlor in the test system, thus implying that bacteria may adsorb pesticides selectively with preference for certain chemical structures over others.

RÉSUMÉ

De nombreux pesticides peuvent former des résidus liés (résidus non récupérés par les techniques courantes d'extraction au soxhlet) après leur application, ce qui augmente leur persistance dans l'environnement. On a effectué une expérience en laboratoire pour étudier le rôle des microorganismes dans la production de ces résidus liés. Pour ce faire, on a incubé de la cyanazine (2-[[4-chloro-6-(éthylamino)-1,3,5-triazin-2-yl]-amino]-2-méthylpropanenitrile) et du métolachlore (2-chloro-N-(2-éthyl-6-méthylphényl)-N-(2-méthoxy-1-méthyléthyl)acétamide) en anaérobiose dans un milieu de culture ayant été inoculé avec une culture bactérienne qui dégrade les HAP. À partir des analyses par CG d'extraits provenant de la dégradation et de l'adsorption, et de contrôles abiotiques pour l'herbicide de départ et ses métabolites éventuels, cette étude a fourni la première preuve directe que la biomasse bactérienne, plutôt que le métabolisme, est principalement responsable de la formation de résidus liés avec la cyanazine. Toutefois, les bactéries anaérobies semblaient incapables de former des résidus liés avec le métolachlore. Le phénomène courant de l'extractibilité selon l'âge des résidus liés a également été observé dans le cas de la cyanazine. L'adsorption apparemment irréversible de la cyanazine par la biomasse bactérienne n'était pas influencée par la présence de métolachlore dans le système d'essai, ce qui implique que les bactéries peuvent adsorber les pesticides de manière sélective et se lier de préférence à certaines substances chimiques plutôt qu'à d'autres.

Introduction

It is often observed that a portion of pesticide residues remains in soil or sediment after exhaustive solvent extraction. These unextracted residues are referred to as "bound residues" (Khan and Hamilton, 1980; Jones *et al.*, 1982). Many commonly used pesticides (e.g., atrazine, fenitrothion, glyphosate, and trifluralin) can form bound residues after application, thus greatly reducing their mobility and increasing their environmental persistence (Helling and Krivonak, 1978; Spillner *et al.*, 1979; Khan and Behki, 1990; Piccolo *et al.*, 1994). In addition, recent studies have also revealed that under given circumstances (e.g., enhanced microbial activity) some of the bound residues can be released from soil (i.e., they are bioavailable) and become absorbed by plants (Khan, 1990; Khan and Behki, 1990). This complicates the issue of pesticide management in the environment, because the real significance of bound residues on ecosystem health is mainly addressed in terms of their bioavailability, both in amount and form of uptake (Helling and Krivonak, 1978). The reported half-lives for the herbicide atrazine in agricultural soils ranged from 37 days (Dao *et al.*, 1979) to as long as 3 to 5 years (Armstrong *et al.*, 1967), probably reflecting the effect of extractability and/or nonextractability for atrazine in environmental samples.

The adsorption of pesticides in natural environments is extremely complicated, involving many physical, chemical, and biological processes/factors (Grover, 1989), such as adsorption by organic matter and clay minerals (Carringer *et al.*, 1975; Barriuso *et al.*, 1992), binding to humic acid and dissolved organic matter (Clay *et al.*, 1988), and formation of pesticide metabolites which are more tightly bound to soil than the parent compounds (Katan and Lichtenstein, 1977). Microorganisms are mainly responsible for the transformation of pesticides in the environment (Somasundaram and Coats, 1991) and are, therefore, directly or indirectly involved in the formation of bound residues (Cheng, 1990). For example, covalent coupling of pesticide metabolites to cellular constituents has been proposed as a cause for the formation of bound residues (Schocken and Speedie, 1984). The irreversible binding of cyanazine metabolites to clay and insoluble organic fractions in soil has been suggested as a major mechanism for the formation of bound residues (Clay et al., 1988). In this communication we demonstrated that bacterial biomass rather than their metabolism was mainly involved in the irreversible adsorption of cyanazine (bound residues) by anaerobic bacteria. The study of cyanazine and metolachlor binding by anaerobic bacteria is important for determining their ultimate fate in sediments where anaerobic conditions may prevail.

Materials and Methods

Chemicals

Analytical grade cyanazine (2-[[4-chloro-6-(ethylamino)-1,3,5,-triazin-2-yl]-amino]-2-methylpropanenitrile) was obtained from Caledon Laboratories, Georgetown, Ont. Reagent grade (97%) metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide] was purchased from Chromatographic Specialties Inc., Brockville, Ont. Pesticide grade organic solvents were obtained from Caledon Laboratories, Georgetown, Ont. The sodium sulfate used for drying organic extracts was heated to 500

°C for 24 h before use. All glassware was also rinsed with pesticide grade solvents before use. All other chemicals used in the experiments were reagent grade or better. The molecular structures of cyanazine and metolachlor are shown in Fig. 1.

Microorganisms

The anaerobic biodegradability of cyanazine and metolachlor was tested with a mixed bacterial culture which had previously been shown to rapidly degrade low and medium molecular weight polynuclear aromatic hydrocarbons (PAHs) and polynuclear aromatic nitrogen heterocycles (Liu *et al.*, 1992). All experiments were conducted anaerobically in 100-mL hypovials over an extended incubation period (up to 6 months) to determine the resistance of these two herbicides to bacterial degradation, and the bioadsorption of the herbicides by bacterial biomass. Experimental procedures are described in detail below.

Growth medium and culture conditions

A growth medium containing the following was prepared (per liter): 5 g each of glucose and sodium acetate, 2 g each of peptone and yeast extract, 100 mL of 10X mineral medium, and 900 mL of distilled water. The 10X mineral medium was prepared by dissolving the following ingredients into 1 L of distilled water (1.3 g of K_2HPO_4, 0.8 g of KH_2PO_4, 0.05 g of $MgSO_4$, 0.5 g of NH_4NO_3, and 0.01 g of $FeSO_4$). The growth medium had a final pH of 6.8 and was not sterilized.

A typical experiment for the adsorption of cyanazine, and/or metolachlor was run as follows: an aliquot of 45 mL of the growth medium was added to each of the 100-mL hypovials, followed by the addition of 100 :L of a freshly anaerobic grown PAHs-degrading bacterial culture (0.92 O.D. at 650 nm) as a source of inoculum. The vials were then purged with a gas mixture of 30% CO_2 and 70% N_2 for 1 min and sealed immediately. The inoculated hypovials were allowed to grow for 4 days (for biomass production) at which point 400 :L of $HgCl_2$ (0.024 g/mL) were injected into each of the hypovials to kill the living cells. The resulting dead cells were used as an adsorption source for the test herbicide. After another 4 days of incubation the hypovials were spiked with cyanazine (in methanol), and/or metolachlor at a final concentration of 10 ppm using a micro syringe.

Biodegradation vials were also prepared in the same manner, with the exception that no $HgCl_2$ was added to the test system. For the abiotic control vials, all the ingredients (growth medium, cyanazine, bacterial inoculum, and $HgCl_2$) were added at the zero hour. All vials were sampled at scheduled time intervals and analyzed for cyanazine, metolachlor, and their possible metabolites.

Sample preparation and chemical analysis

The analysis of cyanazine, and/or metolachlor in the hypovials involved the addition of 20 mL of dichloromethane (DCM) to each vial as it was sampled. The vial was recapped and vigorously hand-shaken for 1 min (neutral extraction). Additional acidic and basic extractions were also performed on the hypovial contents in an attempt to isolate any metabolites that could not be extracted under neutral conditions. The acidic extraction was

performed after the neutral extraction by adjusting the pH of the samples to less than 3 with 1 N HCl that had been previously washed with DCM. Then each of the samples was extracted with 2 x 10 mL DCM, and the resulting extract was dried through anhydrous sodium sulfate, concentrated, and solvent exchanged into toluene (final volume 0.5 mL). The remaining samples (after neutral and acidic extractions) were adjusted to a pH greater than 10 with 1 N NaoH that had also previously been washed with DCM. Then each sample was extracted with 1 x 20 mL and 2 x 10 mL DCM, and the resulting basic extract was dried through anhydrous sodium sulfate before concentrating and solvent exchanging into toluene.

The toluene extracts were analyzed on a Hewlett Packard 5890-II gas chromatograph with a single splitless injector - dual column - nitrogen-phosphorus/flame ionization detector (NPD/FID) technique. Both columns were DB-5 [polymethyl(5% phenyl)siloxane] (J & W Scientific - Chromatographic Specialties Inc., Brockville, Ont.), 0.25 mm i.d. x 30 m length, with 0.25 Fm film thickness. Injector and detector temperatures were 200 °C and 300 °C, respectively. The initial column temperature was 80 °C for 2 minutes, and the program rate was 10 °/minute to 150 °C, then 4 °/minute to 280 °C, and then 8 °/minute to 300 °C with no final hold. The constant helium carrier gas flow rate was 1.0 mL/min. The gas flow rate for air and hydrogen was based on the type of detector used (FID, air - 400 mL/min, hydrogen - 30 mL/min ; NPD, air - 120 mL/min, hydrogen - 4 mL/min). All subsequent mass spectral analyses were performed using the same temperature program and column stationary phase with a Hewlett Packard 5971A gas chromatograph - mass selective detector.

Results and Discussion

About 23% of the spiked cyanazine (9.5 mg/L) had disappeared from the hypovials of the abiotic control after 70 days of anaerobic incubation, as shown in Figure 2. Since this control contained only growth medium, cyanazine, $HgCl_2$ (microbial inhibitor), and a small amount of dead cells (90 :g dry wt/hypovial) introduced by the inoculum and killed by $HgCl_2$ in the reaction mixture, it would appear that factor(s) other than ones which are biological in nature were probably involved in the disappearance of the 23% of the spiked cyanazine in the abiotic control. Loss of cyanazine by volatilization was impossible, because all the hypovials were tightly capped during the entire period of incubation. Moreover, cyanazine has a low vapour pressure at room temperature (Worthing and Walker, 1987) making volatilization unlikely. Experiments by Lau et al. (1996) have also shown that cyanazine can be considered as nonvolatile. Photodegradation of cyanazine in the brown-coloured hypovials was not possible either. Thus, it was deduced that an irreversible adsorption of cyanazine by certain ingredients (possibly organic nutrients and dead bacterial cells) was probably involved in the disappearance of cyanazine in the abiotic control hypovials.

Like other triazine herbicides, cyanazine tends to be strongly adsorbed in soil systems and the binding (adsorption) gradually becomes irreversible over time (Gamerdinger et al., 1991). Many factors, e.g., soil pH (Blumhorst and Weber, 1992), content of clay and insoluble organic fraction (Clay et al., 1988), and formation of metabolite (Clay et al., 1988), could all affect this binding process. During the study of atrazine degradation by higher marine fungi, Schocken and Speedie (1984) found some dealkylated and dechlorinated

products in their uninoculated controls and they attributed the formation of such degradation products to the chemical hydrolysis of atrazine. However, in our experiments no cyanazine degradation products were detected in the abiotic controls, thus eliminating the possible involvement of cyanazine hydrolysis in the disappearance of the spiked cyanazine in the abiotic control (data not shown). Based on all the above evidence, it was tentatively concluded that adsorption by microbial biomass was probably responsible for the disappearance of the spiked cyanazine in the abiotic control hypovials. This conclusion was supported by another adsorption experiment in which a 1/10 strength growth medium (i.e., less bacterial biomass production) was found to result in a slower and incomplete adsorption of the spiked cyanazine (Figure 3).

Caution must be exercised in interpreting the data for any biodegradation experiment, and this is clearly shown in Figure 2. Without the adsorption hypovials (i.e., with the $HgCl^2$ addition to inhibit the microbial degradation process) as a reference, one is tempted to conclude that the disappearance of the spiked cyanazine in the degradation hypovials (i.e., without the $HgCl^2$ addition to retard the microbial degradation process) was primarily due to anaerobic bacterial degradation. However, from Figure 2, it can be seen that the profile of cyanazine disappearance from the degradation hypovials (with live bacteria) mirrored almost exactly that of the adsorption hypovials (with dead bacteria). This strongly indicates that bacterial biomass (regardless of whether alive or dead) rather than metabolism was mainly involved in the disappearance of the spiked cyanazine in the degradation and adsorption hypovials. This conclusion is further supported by the evidence that no trace of cyanazine metabolites had been detected in the neutral, acidic, and basic extractions (DCM) from the degradation, adsorption, and abiotic control hypovials during the entire 70 days of incubation. Thus, the irreversible binding of cyanazine by the alive bacterial biomass (in degradation hypovials) or dead cells (in adsorption hypovials) appears to offer the best explanation for the disappearance of the spiked cyanazine in the degradation and adsorption hypovials. The phenomenon of irreversible binding of cyanazine by dead bacterial cells could also satisfactorily explain the disappearance of some of the spiked cyanazine in the abiotic control hypovials. From Figure 2, it can be seen that the irreversible binding of cyanazine to the bacterial biomass is a time-dependent process. After a 50-days incubation (i.e., contact time between cyanazine and bacterial biomass) all the spiked cyanazine in the degradation and adsorption hypovials had become nonextractable residues. The phenomenon of age-dependent irreversibility for the triazine herbicide residues has been noted in soil systems, with cyanazine (Clay et al., 1988), prometryn (Khan, 1982), and atrazine (Winkelmann and Klaine, 1991).

The irreversible adsorption by the anaerobic bacteria, observed in the experiments with cyanazine, was not observed in the experiments with metolachlor. Results from those experiments (Figure 4) show that anaerobic bacteria, whether alive or dead, could not irreversibly bind metolachlor. After a 90-days incubation, more than 90% of the spiked metolachlor could still be recovered from the degradation and adsorption hypovials. This is vastly different from the adsorption behaviour for cyanazine, for which nearly all the spiked cyanazine had become nonextractable after only a 50-days incubation. Metolachlor appeared to be very stable under the anaerobic condition, as indicated by the absence of its metabolites in the neutral, acidic, and basic extractions from the hypovials.

To assess whether the irreversible adsorption of cyanazine by anaerobic bacteria would

be affected by the presence of another pesticide, the mixed bacterial culture was anaerobically grown for 4 days and a chemical mixture containing cyanazine, metolachlor, and $HgCl^2$ was injected into each of the hypovials. Incubation of the hypovials was then resumed and at selected time intervals a number of hypovials were sacrificed for the analysis of cyanazine and metolachlor (Figure 5). The results clearly indicate that the irreversible adsorption of cyanazine by bacterial biomass was not affected by the presence of metolachlor. This implies that anaerobic bacteria adsorbed pesticides selectively with preference for certain types over others. The experiments were repeated twice and the results, given in terms of the amount of adsorption, (Figure 6) mirrored exactly the previous ones (Figure 5), with less than 5% of the spiked metolachlor adsorbed irreversibly by the bacterial biomass, while almost 100% of the spiked cyanazine had become totally nonextractable after approximately 50-days incubation. Pignatello and Huang (1991) studied the reversible adsorption of atrazine and metolachlor residues in field soils and concluded that there was no competitive sorption between these two herbicides.

Although the formation of bound (nonextractable) pesticide residues in soil had been known to occur for over two decades, their real significance had been critically addressed only recently when it became obvious that these residues were not excluded from environmental interactions (Khan, 1991). Moreover, from the perspective of environmental health, bound residues in most cases could not be detected in the routine analysis involving solvent extraction, and this may lead to an underestimation of the level of pesticides in the environment (Khan and Hamilton, 1980). The mechanism of the formation of bound residues in the environmental matrix is indeed very complicated (Koskinen and Harper, 1990), particularly in view of the fact that the parent compound had been found to constitute a considerable proportion of the bound residues (Khan and Hamilton, 1980). The present study provides the first direct experimental evidence for the time-dependent irreversible adsorption of cyanazine by bacterial biomass. However, we have no explanation as to why the bacterial biomass (alive or dead) irreversibly adsorbed cyanazine but not metolachlor. The phenomenon of the age-dependent extractability of the bound residues for atrazine and metolachlor in agricultural soils has been reported, and the continued diffusion of these herbicides into more remote or "stronger" binding sites in the soil matrix after prolonged incubation was suggested as the cause for the observed time-dependent irreversibility (Pignatello and Huang, 1991). However, such an explanation appeared not applicable to our results, because we used a simpler test system (hypovial) with more chemically defined components (medium) in our adsorption study.

Acknowledgement

This work was support in part by the PESTMYOP and Great Lakes 2000 funds of the Department of Environment.

References

Armstrong, D.E., C. Chesters, and R.F. Harris. 1967. Atrazine hydrolysis in soil. Soil Sci. Soc. Am. Proc. 31:61-66.

Barriuso, E., U. Baer and R. Calvet. 1992. Dissolved organic matter and adsorption-

desorption of dimefuron, atrazine, and carbetamide by soils. J. Environ. Qual. 21:359-367.

Carringer, R.D., J.B. Weber and T.J. Monaco. 1975. Adsorption-desorption of selected pesticides by organic matter and montmorillonite. J. Agri. Food Chem. 23:568-572.

Clay, S.A., R.R. Allmaras, W.C. Koskinen and D.L. Wyse. 1988. Desorption of atrazine and cyanazine from soil. J. Environ. Qual. 17:719- 723.

Cheng, H.H. 1990. Pesticides in the Soil Environment:Processes, Impacts, and Modeling. Soil Sci. Soc. Am., Madison, 530pp.

Dao, T.H. and T.L. Lavy. 1978. Atrazine adsorption on soil as influenced by temperature, moisture content, and electrolyte concentration. Weed Sci. 26:303-308.

Gamerdinger, A.P., A.T. Lemley, and R.J. Wagenet. 1991. Nonequilibrium sorption and degradation of three 2-chloro-*s*-triazine herbicides in soil-water systems. J. Environ. Qual. 20:815-822.

Grover, R. 1989. Environmental Chemistry of Herbicide, volume 1, CRC press, Boca Raton, 207pp.

Harvey, Jr.J. and J.C.Y. Han. 1978. Decomposition of oxamyl in soil and water. J. Agri. Food Chem. 26:536-541.

Hayes, M.H.B. 1970. Adsorption of triazine herbicides on soil organic matter, including a short review on soil organic matter chemistry. Residue Rev. 32:131-174.

Helling, C.S. and A.E. Krivonak. 1978. Biological characteristics of bound dinitroaniline herbicides in soils. J. Agri. Food Chem. 26:1164-1172.

Hunter, C. and B. McGee. 1994. Survey of pesticide use in Ontario, 1993. Economics Information Report No. 94-01. Ontario Ministry of Agriculture, Food and Rural Affairs, Toronto, Ontario, 31 pp.

Jones, T.W., W.M. Kemp, J.C. Stevenson and J.C. Means. 1982. Degradation of atrazine in estuarine water/sediment systems and soils. J. Environ. Qual. 11:632-638.

Katan, J. and E.P. Lichtenstein. 1977. Mechanisms of production of soil- bound residues of [^{14}C]parathion by microorganisms. J. Agri. Food Chem. 25:1404-1408.

Kaufman, D.D. and P.C. Kearney. 1970. Microbial degradation of *s*-triazine herbicides. Residue Rev. 32:235-265.

Khan, S.U. and H.A. Hamilton. 1980. Extractable and bound (nonextractable) residues of prometryn and its metabolites in an organic soil. J. Agric. Food Chem. 28:126-132.

Khan, S.U. 1982. Distribution and characteristics of bound residues of prometryn in an

organic soil. J. Agric. Food Chem. 30:175-179.

Khan, S.U. and R.M. Behki. 1990. Effect of *Pseudomonas* species on the release of bound ^{14}C residues from soil treated with [^{14}C]atrazine. J. Agric. Food Chem. 38:2090-2093.

Khan, S.U. 1991. Bound (nonextractable) pesticide degradation products in soils:Bioavailability to plants. In Somasundaram, L. and J.R. Coats (eds.), Pesticide Transformation Products: Fate and Significance in the Environment, Am. Chem. Soc., Washington, D.C., pp 109-121.

Koskinen, W.C., and S.S. Harper. 1990. The retention process:Mechanism. In H.H. Cheng (ed.), Pesticides in the Soil Environment:Processes, Impacts, and Modeling. Soil Sci. Soc. Am., Madison, pp51-77.

Lau, Y.L., Liu, D. and Pacepavicius, G.J. 1996. A preliminary study of the persistence of cyanazine in the aquatic environment. NWRI Contribution 96-155.

Liu, D., R.J. Maguire, G.J. Pacepavicius and E. Nagy. 1992. Microbial degradation of polycyclic aromatic hydrocarbons and polycyclic aromatic nitrogen heterocyclics. Environ. Toxicol. Water Qual. 7:355-372.

Pauli, B.D., R.A. Kent and M.P. Wong. 1991. Canadian Water Quality Guidelines for Cyanazine. Department of the Environment, Inland Waters Directorate Scientific Series No. 180, Ottawa, ISBN 0-662-18512-9, 26 pp.

Piccolo, A., G. Celano, M. Arienzo and A. Mirabella. 1994. Adsorption and desorption of glyphosate in some European soils. J. Environ. Sci. Health. B29:1105-1115.

Pignatello, J.J. and L.Q. Huang. 1991. Sorptive reversibility of atrazine and metolachlor residues in field soil samples. J. Environ. Qual. 20:222-228.

Schocken, M.J. and M.K. Speedie. 1984. Physiological aspects of atrazine degradation by higher marine fungi. Arch. Environ. Contam. Toxicol. 13:707-714.

Singh, G., W.F. Spencer, M.M. Cliath, and M.T. van Genuchten. 1990. Sorption behavior of s-triazine and thiocarbamate herbicides on soils. J. Environ. Qual. 19:520-525.

Somasundaram, L. and J.R. Coats. 1991. Pesticide Transformation Products: Fate and Significance in the Environment. Am. Chem. Soc., Washington, D.C., 305pp.

Spillner, Jr., C.J., J.R. DeBaun and J.J. Menn. 1979. Degradation of fenitrothion in forest soil and effects on forest soil microbes. J. Agric. Food Chem. 27:156-160.

Winkelmann, D.A. and S.J. Klaine. 1991. Atrazine metabolite behavior in soil-core microcosms:formation, disappearance, and bound residues. In L. Somasundaram and J.R. Coats (eds.), Pesticide Transformation Products: Fate and Significance in the Environment, Am. Chem. Soc., Washington, pp 75-91.

Worthing, C.R., and S.B. Walker. 1987. The Pesticide Manual: A World Compendium, 8th ed. Thornton Heath, U.K., British Crop Protection Council. 1081 pp.

Yu, C.C., G.M. Booth and J.R. Larsen. 1975. Fate of triazine herbicide cyanazine in a model ecosystem. J. Agric. Food Chem. 23:1014-1015.

Figure Captions

Fig. 1. Chemical structures of cyanazine and metolachlor.

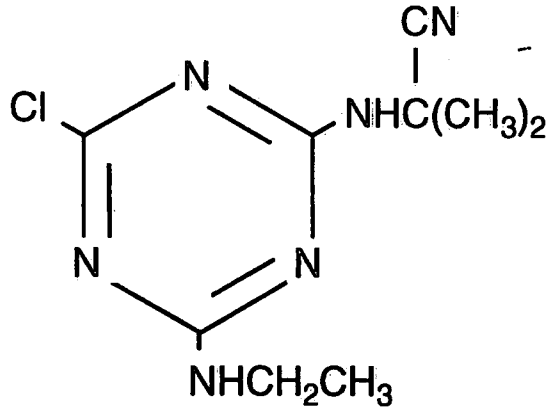
Fig. 2. Concentration profiles of spiked cyanazine in anaerobic hypovials.

Fig. 3. Concentration profile of spiked cyanazine in 1/10 strength growth medium (note the slower and incomplete cyanazine adsorption due to lower biomass).

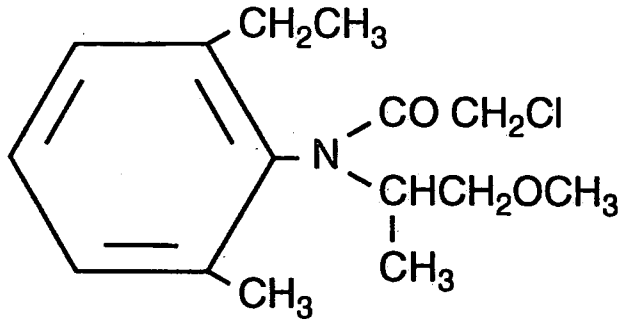
Fig. 4. Concentration profiles of spiked metolachlor in anaerobic hypovials.

Fig. 5. Competitive effect of cyanazine and metolachlor on their adsorption behaviour by bacterial biomass.

Fig. 6. Time course of cyanazine and metolachlor adsorption by bacterial biomass.



Cyanazine



Metolachlor

Figure 1

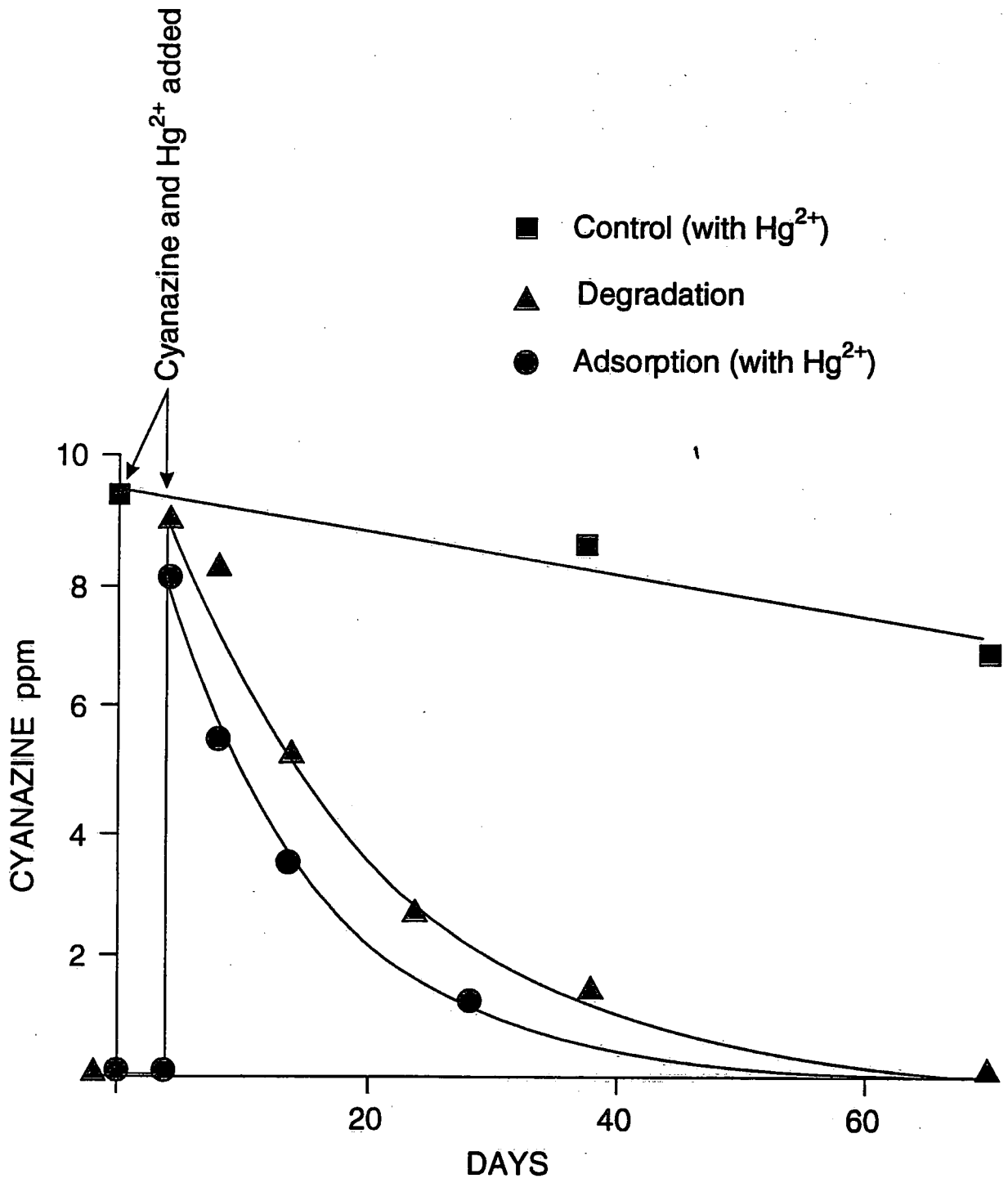

Cyanazine and Hg^{2+} added
Control (with Hg^{2+})
Degradation
Adsorption (with Hg^{2+})
CYANAZINE ppm
10
8
6
4
2
20
40
60
DAYS

Figure 2

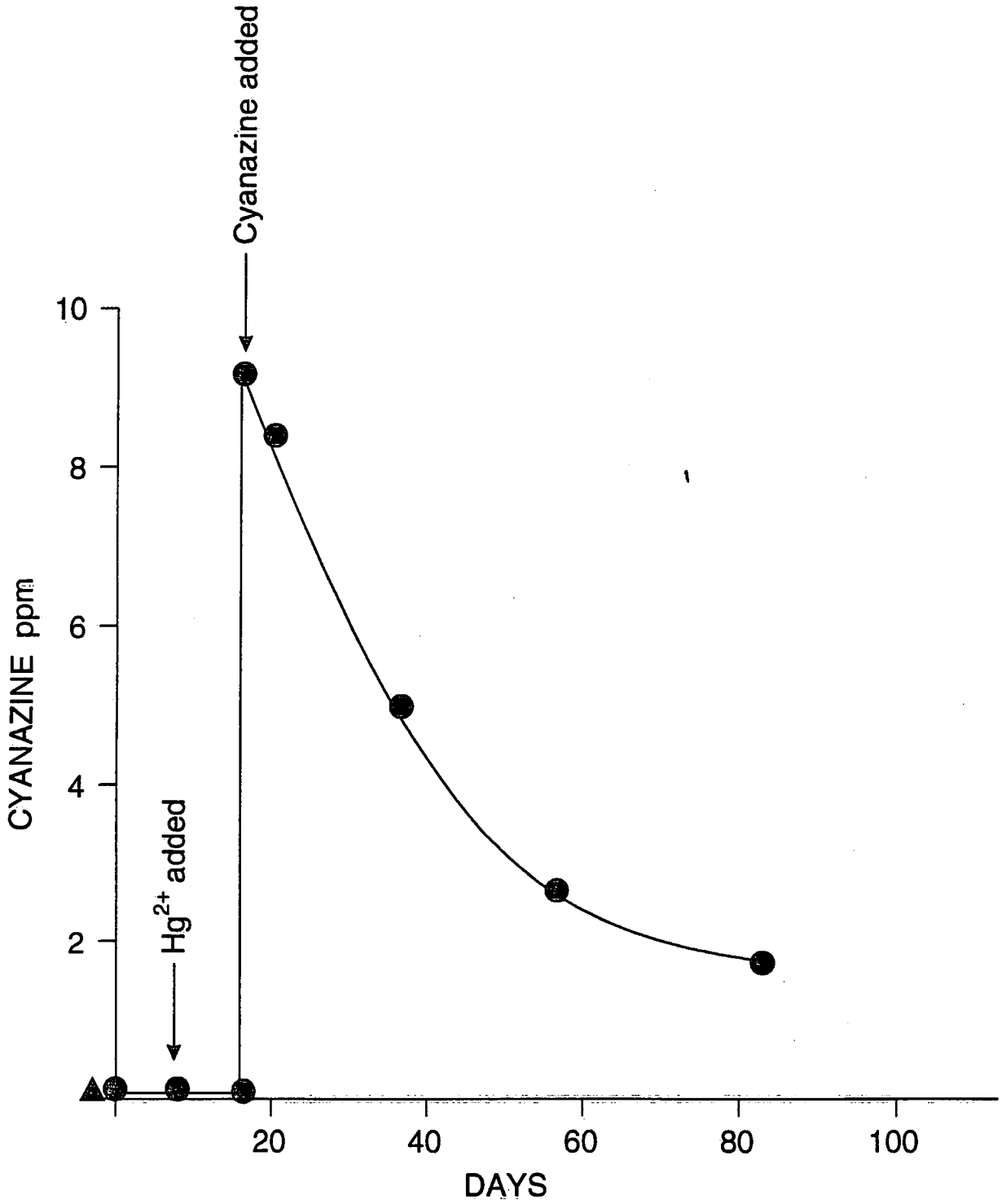

Cyanazine added
10
8
6
4
2
CYANAZINE ppm
Hg^{2+} added
20
40
60
80
100
DAYS

Figure 3

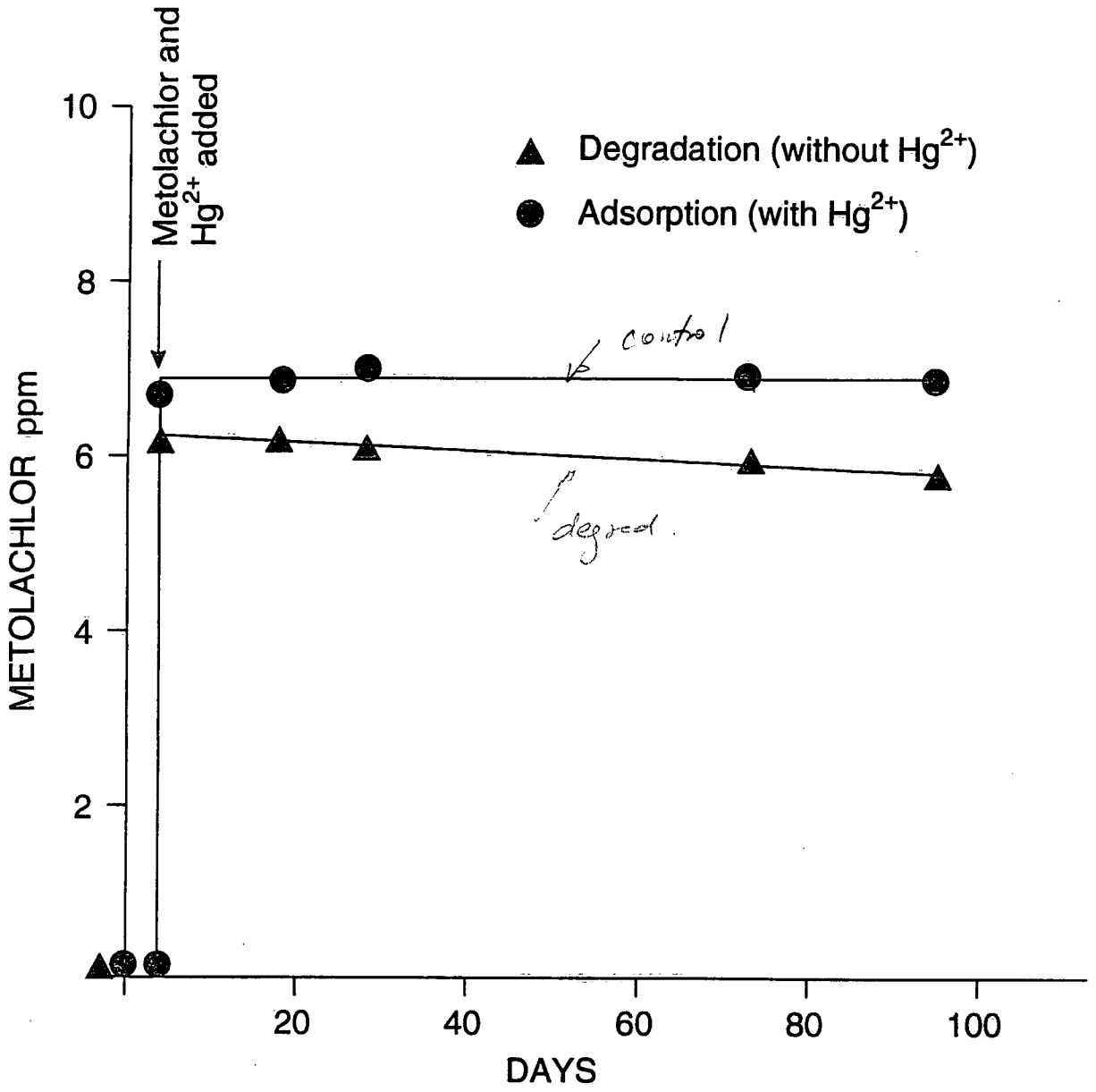

Metolachlor and Hg^{2+} added
Degradation (without Hg^{2+})
Adsorption (with Hg^{2+})
control
degrad.
METOLACHLOR ppm
10
8
6
4
2
20
40
60
80
100
DAYS

Figure 4

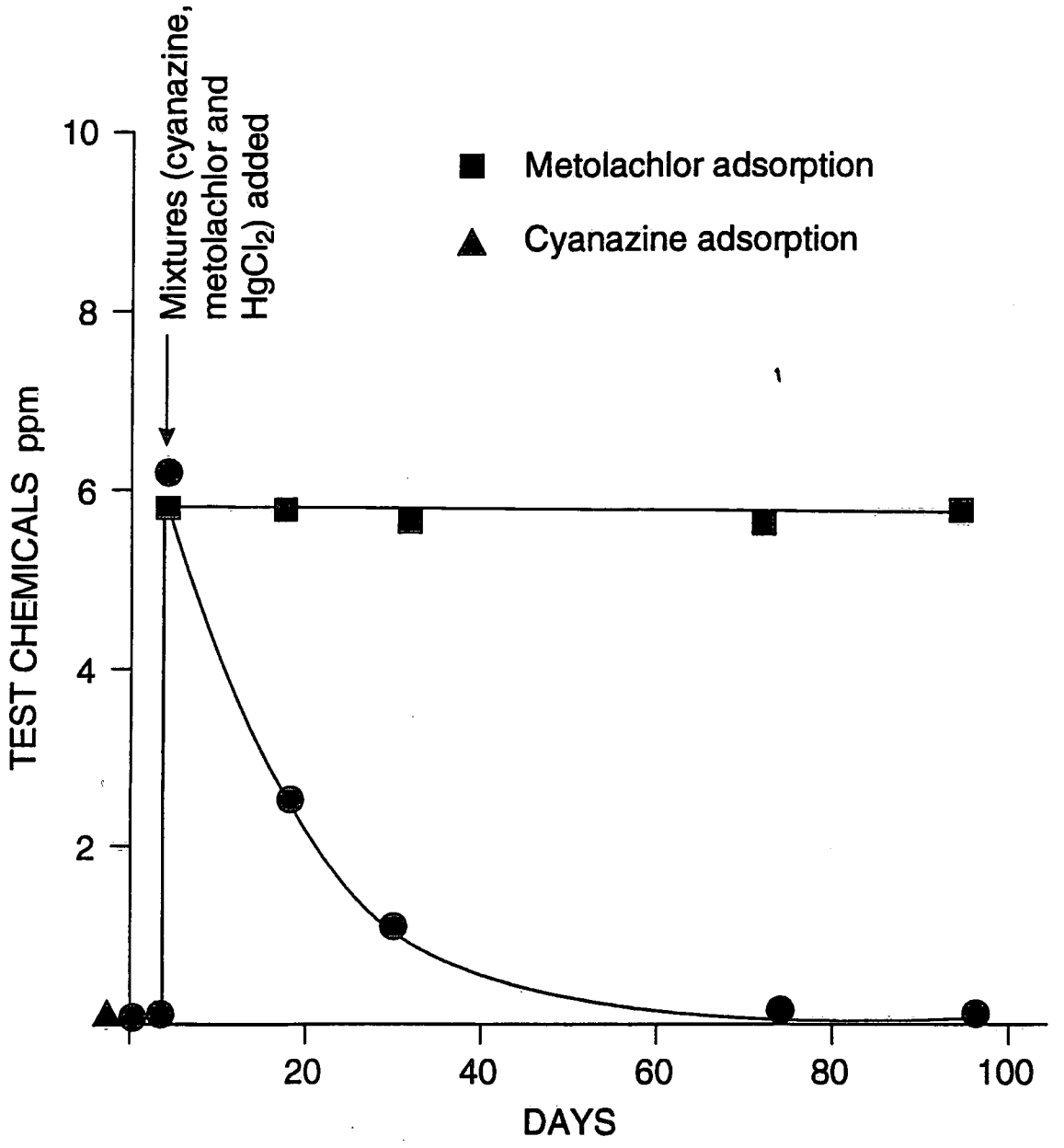

Mixtures (cyanazine, metolachlor and $HgCl_2$) added
Metolachlor adsorption
Cyanazine adsorption
TEST CHEMICALS ppm
10
8
6
4
2
20
40
60
80
100
DAYS

Figure 5

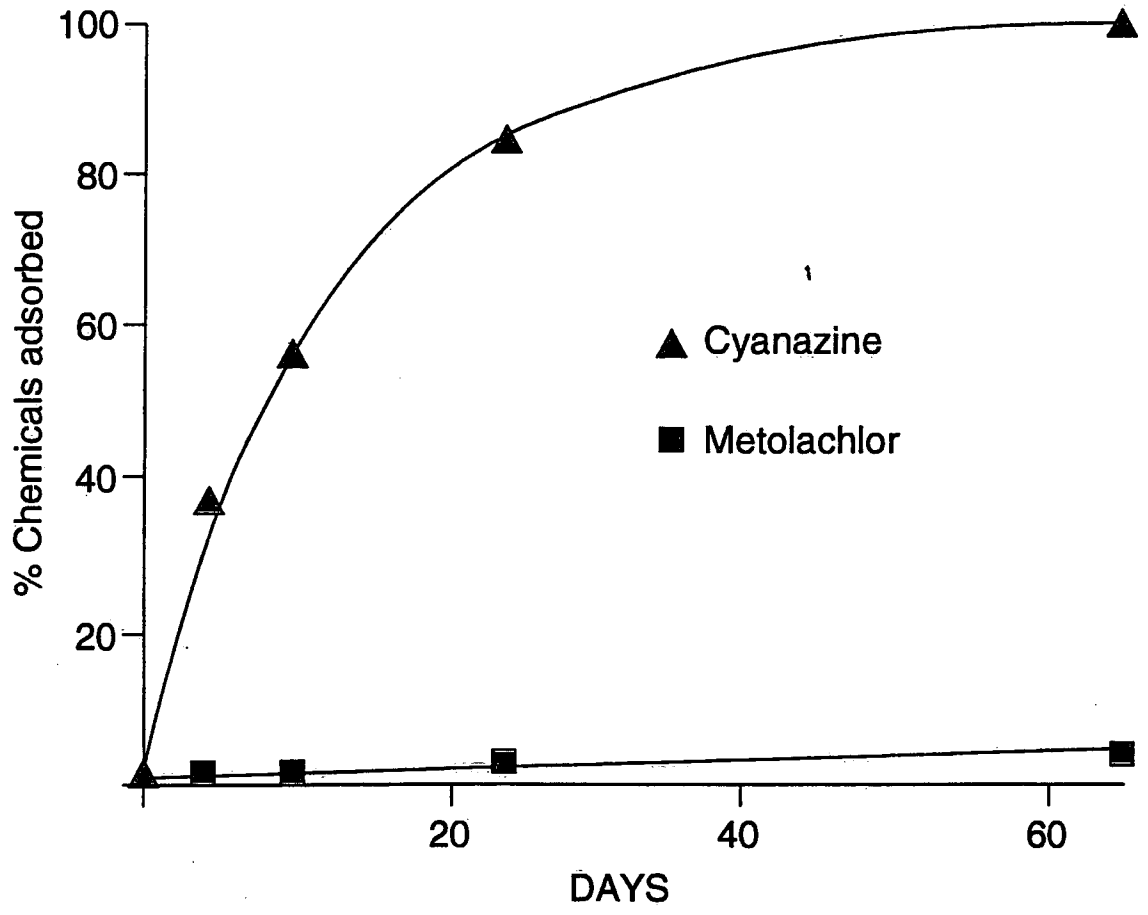
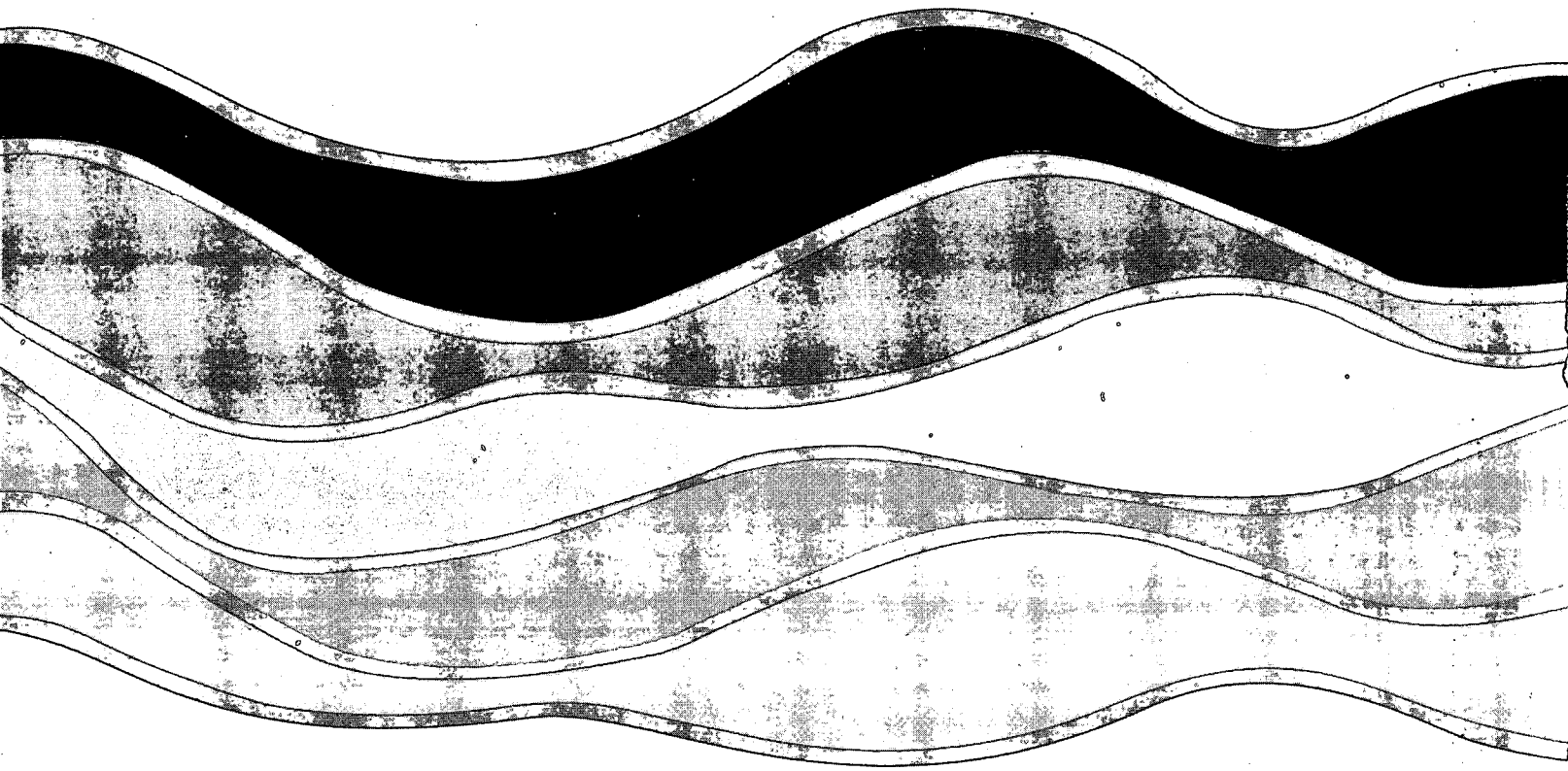

100
80
60
40
20
% Chemicals adsorbed
Cyanazine
Metolachlor
20
40
60
DAYS

Figure 6



NATIONAL WATER RESEARCH INSTITUTE
P.O. BOX 5050, BURLINGTON, ONTARIO L7R 4A6

Environment Canada Environnement Canada

Canada

INSTITUT NATIONAL DE RECHERCHE SUR LES EAUX
C.P. 5050, BURLINGTON (ONTARIO) L7R 4A6

Think Recycling!

Pensez à recycler !