98-001

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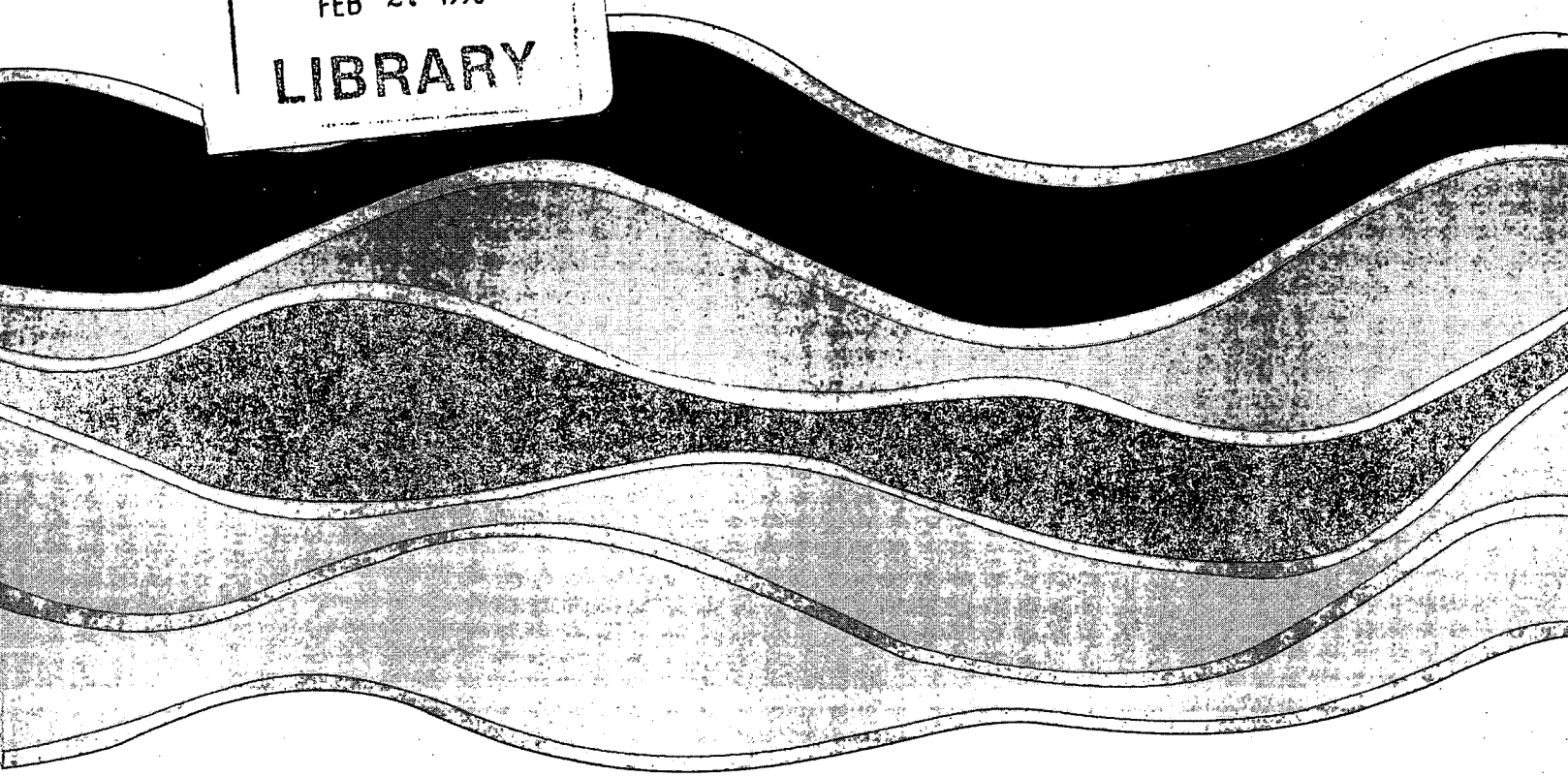

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NWRI Contribution No. 98-001

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by

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MANAGEMENT PERSPECTIVE

Irgarol 1051, 2-methylthio-4-tert-butylamino-6-cyclopropylamino-s-triazine, is a newly developed herbicidal additive for use in copper-based antifouling paints. It is intended to replace the widely used antifouling agent tributyltin (TBT) which has been regulated internationally since 1990, primarily due to its severe impact on the aquatic ecosystem. For example, it has been estimated that, in the Arcachon Bay of France alone, the use of TBT caused a loss in revenue of 147 million U.S. dollars through reduced oyster production. Very recent studies have shown that TBT can cause sexual changes in gastropods. Females, upon exposure to levels in parts per trillion range, developed male characteristics such as penises (the so-called imposex problem), thus seriously reducing the population of gastropods in the marine environment.

The use of a suitable herbicide such as Irgarol 1051 in copper-based antifouling paints is necessary, because copper salts themselves are not effective in inhibiting the primary colonization of a ship's hull surface by micro-algae and subsequent growth of seaweed. Fouling on ships increases hull roughness leading to larger frictional resistance and increased fuel consumption. It has been estimated that a 1-mm thick slime layer on the hull could cause an 80% increase in surface friction and 15% loss in ship speed. Therefore, the use of antifouling paint on ships has very important economic implications. Irgarol is a new chemical and, until very recently, there was no information in the open literature on the persistence and degradation: a fact that hinders the assessment of its ultimate impact on the environment. Our laboratories were the first ones to successfully work out the pathway for the microbial transformation of Irgarol 1051. Canada is a country with a very long coast line. Therefore, it is essential to understand the possible impact of a new chemical on our aquatic environment. For this reason, Irgarol 1051 has been one of the priority chemicals being investigated in our laboratories since 1995. This report summarizes our investigation on the abiotic degradation of Irgarol 1051 by mercuric chloride.

This study showed that the inorganic salt mercuric chloride could effect a rapid and quantitative hydrolysis of Irgarol 1051 in distilled water and buffer solutions. The reaction was not significantly affected by the ambient pH (5 - 9) for most natural waters. The degradation also appeared to follow the reaction of a catalyzed hydrolysis. Specific activity (for Hg^{2+}) was apparently critical in this catalyzed hydrolysis, because none of the other 5 heavy metal salts tested ($AgNO_3$, $CdCl_2$, $CuSO_4$, $PbCl_2$, and $ZnCl_2$) had any catalytic property on Irgarol hydrolysis. The mechanism of the catalyzed hydrolysis may be the formation of bidentate chelation through nitrogen (No. 5) on the ring and the nitrogen on the cyclopropylamino side chain in Irgarol 1051 with the mercuric ion. The resulting four-member chelate complex would weaken the cyclopropyl-amino bond considerably, thus facilitating the hydrolysis reaction. Ultraviolet spectroscopy of the reaction mixture and the positive identification of Irgarol hydrolysis product M1 by GC-MS and LC-MS provided the basis for the proposed mechanism on $HgCl_2$-catalyzed hydrolysis of Irgarol 1051. M1 appeared to be more stable than the parent compound Irgarol 1051, thus implying its possible accumulation in the environment.

Irgarol 1051 is not presently registered for use in Canada under the Pest Control Products Act. In anticipation of its registration, and because it is registered in some other countries, including the United States, we are presently determining whether it is present in several large harbours in Canada and Japan which can occur from the leaching of ships painted in other countries. In the

meantime, we are broadening the research on the mechanisms and processes involved in Irgarol degradation to further our understanding and knowledge about the impact of this new chemical on the aquatic environment. This research includes biological, chemical, and photo degradation of Irgarol 1051.

It may be appropriate to note here the rationale for our attempts to identify degradation products in various reaction mixtures. Degradation products, sometimes, can be more toxic, mobile, and/or persistent than the parent compound. A good example is hydroxyatrazine, an atrazine metabolite. This compound has a much greater affinity for soil and sediment than does the parent compound. Therefore, formation of hydroxyatrazine could significantly affect our estimation of atrazine's environmental fate and persistence. The results from this abiotic study and our previous investigation on Irgarol's biotransformation strongly suggest that M1 (2-methylthio-4-tert-butylamino-6-amino-s-triazine) could be a very stable intermediate and/or terminal end product during the biological and chemical degradation of Irgarol 1051. This information would be very useful in the management of Irgarol 1051 in the aquatic environment.

Probably one of the most important and practical aspects of this work is the demonstration of rapid decompostion of Irgarol 1051 by mercuric chloride. Consequently, $Hgcl_2$ should never be used as a microbial inhibitor in preserving water samples in Irgarol monitoring programs. Mercuric chloride has been widely as microbial inhibitor to control biological degradation in environmental samples including the US EPA National Pesticide Survey Program.

SOMMAIRE À L'INTENTION DE LA DIRECTION

L'Irgarol 1051, c.-à-d. la 2-méthylthio-4-tert-butylamino-6-cyclopropylamino-s-triazine, est un nouvel additif herbicide pour les peintures antiparasitaires à base de cuivre. Il a été conçu pour remplacer le tributylétain (TBE), largement utilisé, et soumis depuis 1990 à une réglementation internationale en raison principalement de ses effets néfastes sur l'écosystème aquatique. Par exemple, on a estimé que dans la seule baie d'Arcachon en France, l'emploi du TBE a entraîné une perte de revenus de 147 millions de $ U.S. par suite de la production réduite d'huîtres. Des études très récentes ont montré que le TBE pouvait provoquer des changements sexuels chez les gastropodes. Chez les femelles, après exposition à des concentrations de plusieurs parties par trillion, il y a eu apparition de pénis (problème de l'imposexe), ce qui a fortement réduit la population de gastropodes dans le milieu marin.

L'emploi d'un herbicide approprié, comme l'Irgarol 1051, est nécessaire dans les peintures antiparasitaires à base de cuivre, car les sels de cuivre tout seuls ne suffisent pas à empêcher la colonisation primaire de la coque d'un navire par les micro-algues et la croissance ultérieure de plantes marines. Les salissures sur les navires augmentent la rugosité de la coque, d'où une plus grande résistance au frottement et une consommation plus importante de carburant. On a évalué qu'une épaisseur de 1 mm de dépôt sur la coque pouvait entraîner un accroissement de 80 % du frottement en surface et une perte de 15 % de la vitesse du navire. L'emploi de peinture antiparasitaire sur les navires a donc des répercussions économiques très importantes. L'Irgarol est un nouveau produit chimique et, jusqu'à tout récemment, les publications ne donnaient aucune information sur sa persistance et sa dégradation, d'où l'impossibilité d'évaluer ses effets ultimes sur l'environnement. Nos laboratoires ont été les premiers à étudier le processus de la transformation microbienne de l'Irgarol 1051. Le Canada est un pays qui possède beaucoup de côtes. Il est donc très important de connaître les effets d'un nouveau produit chimique sur le milieu aquatique. Voilà pourquoi l'Irgarol 1051 a été l'un des produits étudiés en priorité dans nos laboratoires depuis 1995. Le présent rapport résume nos recherches sur la dégradation abiotique de l'Irgarol 1051 par le chlorure mercurique.

La présente étude montre que le chlorure mercurique, sel minéral, permettait d'hydrolyser rapidement et quantitativement l'Irgarol 1051 dans l'eau distillée et les solutions tampons. Dans la plupart des eaux naturelles, la réaction n'était pas sensiblement modifiée de façon significative par le pH ambiant (5 à 9). La dégradation semblait également prendre la forme d'une réaction d'hydrolyse catalysée. L'activité spécifique (pour Hg^{2+}) était très nette dans cette hydrolyse catalysée, en effet aucun des 5 autres sels de métaux lourds expérimentés ($AgNO_3$, $CdCl_2$, $CuSO_4$, $PbCl_2$ et $ZnCl_2$) n'exerçait un effet catalytique sur l'hydrolyse de l'Irgarol. Le mécanisme de l'hydrolyse catalytique peut être la chélation bidentée via l'azote (N° 5) sur le cycle et l'azote de la chaîne latérale cyclopropylamino de l'Irgarol 1051 avec l'ion mercurique. Le complexe chélaté à quatre membres affaiblirait considérablement la liaison cyclopropyl-amino, facilitant ainsi la réaction d'hydrolyse. La spectroscopie UV du mélange réactionnel et l'identification positive du produit M1 de l'hydrolyse de l'Irgarol par CG-SM et CL-SM a permis d'établir la base du mécanisme proposé pour l'hydrolyse de l'Irgarol 1051, catalysée par $HgCl_2$. M1 semblait plus stable que le composé parent Irgarol 1051, ce qui laisse supposer sa possible accumulation dans l'environnement.

Actuellement, l'Irgarol 1051 n'est pas homologué au Canada dans le cadre de la Loi sur les produits antiparasitaires. En attendant son homologation, et vu qu'il est déjà homologué dans certains autres pays, notamment aux États-Unis, nous sommes en train de déterminer si ce produit peut être présent dans divers grands ports du Canada et du Japon par suite de son

lessivage à partir de navires peints dans d'autres pays. Dans l'intervalle, nous élargissons nos recherches sur les mécanismes et processus intervenant dans la dégradation de l'Irgarol, de façon à mieux comprendre et cerner plus étroitement les effets de ce nouveau produit chimique sur le milieu aquatique. Ces recherches comprennent la dégradation biologique et chimique ainsi que la photodégradation d'Irgarol 1051.

Il serait utile de présenter les principes à la base de notre démarche visant à caractériser les produits de dégradation dans divers mélanges réactionnels. Ces produits peuvent parfois être plus toxiques, mobiles et (ou) persistants que le composé parent. Un bon exemple est l'hydroxyatrazine, un métabolite de l'atrazine. Ce composé a une affinité beaucoup plus grande pour les sols et les sédiments que le composé parent. La formation d'hydroxyatrazine pourrait donc influer de façon significative sur notre évaluation du devenir et de la persistance de l'atrazine dans l'environnement. Les résultats de cette étude abiotique et nos recherches antérieures sur la biotransformation de l'Irgarol laissent fortement supposer que le M1 (2-méthylthio-4-tert-butylamino-6-amino-s-triazine) pourrait être un intermédiaire et (ou) un produit final très stable pendant la dégradation biologique et chimique de l'Irgarol 1051. Cette information serait très utile pour la gestion de l'Irgarol 1051 dans le milieu aquatique.

L'un des aspects les plus importants et les plus pratiques de ces travaux est probablement la démonstration de la rapide décomposition de l'Irgarol 1051 par le chlorure mercurique. Le $HgCl_2$ ne devrait jamais être utilisé comme antimicrobien pour la préservation des échantillons d'eau dans les programmes de surveillance de l'Irgarol. Le chlorure mercurique a été largement utilisé comme antimicrobien pour empêcher la dégradation biologique dans les échantillons environnementaux, y compris dans le programme national d'étude des pesticides de l'EPA (États-Unis).

ABSTRACT

Irgarol 1051, 2-methylthio-4-tert-butylamino-6-cyclopropylamino-s-triazine, is a newly developed herbicidal additive for use in copper-based antifouling paints. It is intended to replace the antifouling agent tributyltin, which has been regulated internationally due to its severe impact on the aquatic ecosystem. However, there is no information in the open literature on the abiotic degradation of Irgarol, a fact that hinders the assessment of its ultimate impact on the environment. This study showed that mercuric chloride was capable of rapidly catalyzing the hydrolysis of Irgarol 1051 in distilled water and buffer solutions. The degradation appeared to follow the reaction of a catalyzed hydrolysis, and was not significantly affected by the pH tested (5 to 9). All other 5 heavy metal salts tested ($AgNO_3$, $CdCl_2$, $CuSO_4$, $PbCl_2$, and $ZnCl_2$) had practically no catalytic property on Irgarol hydrolysis, implying the involvement of a specific activity for Hg^{2+} in this reaction. The mechanism for the catalyzed hydrolysis may be the formation of bidentate chelation through nitrogen (No. 5) on the ring and the nitrogen on the cyclopropylamino side chain in Irgarol 1051 with the Hg^{2+} ion. The resulting four-member chelate complex would weaken the cyclopropyl-amino bond considerably, thus facilitating the hydrolysis reaction. Ultraviolet spectroscopy of the reaction mixtures and the identification of Irgarol hydrolysis product M1 (2-methylthio-4-tert-butylamino-6-amino-s-triazine) by GC-MS and LC-MS provided the basis for the proposed mechanism on the $HgCl_2$-catalyzed hydrolysis of Irgarol 1051. M1 appeared to be more stable than the parent compound Irgarol 1051, thus implying its possible accumulation in the environment. One practical aspect of this work is that $HgCl_2$ should not be used in preserving water samples in Irgarol 1051 monitoring programs.

RÉSUMÉ

L'Irgarol 1051, soit la 2-méthylthio-4-tert-butylamino-6-cyclopropylamino-s-triazine, est un nouvel additif herbicide pour les peintures antiparasitaires à base de cuivre. Il a été conçu pour remplacer le tributylétain, soumis à une réglementation internationale en raison de ses effets néfastes sur l'écosystème aquatique. Cependant, les publications ne donnent pas d'information sur sa persistance et sa dégradation, d'où l'impossibilité d'évaluer ses effets ultimes sur l'environnement. La présente étude a montré que le chlorure mercurique catalysait rapidement l'hydrolyse de l'Irgarol 1051 dans l'eau distillée et les solutions tampons. La dégradation semblait prendre la forme d'une réaction d'hydrolyse catalysée, et n'était pas sensiblement modifiée par le pH (5 à 9). Pratiquement aucun des 5 autres sels de métaux lourds expérimentés ($AgNO_3$, $CdCl_2$, $CuSO_4$, $PbCl_2$ et $ZnCl_2$) n'avait un effet catalytique sur l'hydrolyse de l'Irgarol, ce qui laisse supposer un rôle spécifique pour Hg^{2+} dans cette réaction. Le mécanisme de l'hydrolyse catalytique peut être la chélation bidentée via l'azote (N° 5) sur le cycle et l'azote de la chaîne latérale cyclopropylamino de l'Irgarol 1051 avec l'ion Hg^{2+}. Le complexe chélaté résultant à quatre membres affaiblirait considérablement la liaison cyclopropyl-amino, facilitant ainsi la réaction d'hydrolyse. La spectroscopie UV des mélanges réactionnels et l'identification du produit M1 (2-méthylthio-4-tert-butylamino-6-amino-s-triazine) de l'hydrolyse de l'Irgarol, par CG-SM et CL-SM, a permis d'établir la base du mécanisme proposé pour l'hydrolyse de l'Irgarol 1051, catalysée par $HgCl_2$. M1 semble plus stable que le composé parent Irgarol 1051, ce qui laisse supposer sa possible accumulation dans l'environnement. Un des aspects pratiques de ces travaux est que le $HgCl_2$ ne devrait pas être employé pour la préservation des échantillons d'eau dans les programmes de surveillance de l'Irgarol 1051.

INTRODUCTION

Organotin biocides (e.g., tributyltin and triphenyltin) have been widely used in the formulations of antifouling paint. However, their wide environmental distribution (Langston et al., 1990) and non-selective biotoxicity (Beaumont and Newman, 1986) have led the OECD countries in 1988 to restrict their use in antifouling paints (Evans et al., 1995). Since then copper-based biocides, fortified with a herbicide, have largely replaced organotins in antifouling paints worldwide (Readman et al., 1993). Irgarol 1051, 2-methylthio-4-tert-butylamino-6-cyclopropylamino-s-triazine, is a newly developed herbicidal additive for use in copper-based antifouling paints.

Little is known to date about the long-term toxicity and degradability of Irgarol 1051 (Toth et al., 1996). Irgarol 1051 is not considered to be readily biodegradable and its degradation in sea and freshwater is slow, with half-lives of about 100 and 200 days, respectively (Ciba Geigy, 1995). It is also immune to bacterial degradation, but can be biotransformed by the white rot fungus Phanerochaete chrysosporium via the mechanism of N-dealkylation to yield a stable metabolite M1 (Liu et al., 1997). Irgarol 1051 is highly toxic to algae and can cause a significant change in structure of the marine periphyton community at an ambient concentration of 0.063-0.25 ug L^{-1} (Dahl and Blanck, 1996). This concentration is well within the ranges (0.002-0.19 ug L^{-1}) detected in the contaminated areas of the southern England and the coastal waters of France (Dahl and Blanck, 1996). Thus, the continuous use of Irgarol 1051 in antifouling paints will likely damage microalgal communities in contaminated coastal waters.

To better understand the ultimate impact of Irgarol 1051 on the aquatic ecosystem, factors and processes affecting the environmental persistence of this chemical have become one of the major research topics in our laboratories since 1995. In this communication, we report on the rapid hydrolysis of Irgarol 1051 in aqueous solution by mercuric chloride. The possible mechanism involved in the catalyzed hydrolysis of Irgarol 1051 is also discussed.

MATERIAL AND METHODS

Chemicals

Irgarol 1051 [(2-methylthio-4-tert-butylamino-6-cyclopropylamino-s-triazine), identification no. 84611.0] of high grade (95%) was a gift of Ciba-Geigy Canada Ltd., Mississauga, Ontario. All the test inorganic and organic chemicals were obtained from BDH Chemicals (Toronto, Canada). The inorganic salts used in the test experiments included $AgNO_3$, $CdCl_2 \cdot 2 \cdot 5H_2O$, $CuSO_4 \cdot 5H_2O$, $HgCl_2$, $PbCl_2$, and $ZnCl_2$. Pesticide grade organic solvents were obtained from Caledon Laboratories, Georgetown, Ontario. The sodium sulphate used for drying organic extracts was heated to 500°C for 24 h before use. All glassware were also rinsed with pesticide grade solvents before use. All other chemicals used in the experiments were reagent grade or better.

Experimental Procedure

Hydrolysis of Irgarol 1051 was tracked by observing the disappearance of the parent compound and the appearance of its degradation products in the reaction mixture. The measurement was made by using HPLC (high performance liquid chromatography), LC-MS (liquid chromatography mass spectrometry), GC (gas chromatograph), GC-MS (gas chromatography mass spectrometry), and UV (ultraviolet) spectrophotometric analysis. Typically, 5 mg of Irgarol 1051 in 1 L of distilled water or in buffered solution (20 mM ammonium acetate-acetic acid-sodium hydroxide for pH 5.0, 7.0 and 9.0) was treated with $HgCl_2$ (20 mg/L). The reaction mixture was kept at room temperature (21°C) for 2 hours before analyzing for Irgarol 1051 and its hydrolysis products.

To study the effect of various heavy metals (in the salt form) on the hydrolysis of Irgarol 1051, a final concentration of 10, 20, and 100 mg/L of $AgNO_3$, $CdCl_2.2.5H_2O$, $CuSO_4.5H_2O$, $HgCl_2$, MeHgCl, $PbCl_2$, or $ZnCl_2$, was each individually tested in the Irgarol solution (5 mg/L). To assess the concentration effect of mercuric chloride on the hydrolysis of Irgarol 1051, $HgCl_2$ at a final concentrations of 0, 0.1, 1, 10, 20, and 100 mg/L was used. Kinetic studies of hydrolysis were conducted by removing an aliquot (50-100 mL) of the reaction mixtures at different times (0, 1, 10, and 60 min, 2, 6, 24, 48, and 120 hr). The percentages of remaining Irgarol 1051 and the hydrolysis products formed (mainly M1) were measured through GC, GC-MS, HPLC and LC-MS analysis.

Sample Preparation and Chemical Analysis

For the analysis of Irgarol 1051 and its degradation products by GC or GC-MS, an aliquot (100 mL) of the reaction mixture was transferred to a 500-mL separatory funnel containing 50 mL of DCM (dichloromethane). The extraction was repeated once with another 50 mL of DCM, and the combined DCM extracts with a tolune keeper were reduced to 5 mL on a rotary evaporator. Further concentration and solvent exchange into toluene were performed under a nitrogen stream. For analysis by HPLC or LC-MS, the combined DCM extracts were evaporated to dryness and then the residue was re-dissolved into 0.3 mL of MeCN (acetonitrile).

For GC analysis, the toluene extracts were analyzed on a Hewlett Packard 5890 gas chromatograph equipped with a nitrogen-phosphorus detector (300°C) and a flame ionization detector (300°C) utilising an oven program with a 2 min hold at 80°C and a temperature ramp of 10°C/min to 150°C followed by a temperature ramp of 4°C/min to 280°C and a final temperature ramp of 8°C/min to 300°C. The columns used were dual DB5 coated capillary columns (0.25 mm x 27 m) which had been installed into the injector (200°C) in the splitless mode with a constant helium carrier flow of 0.8 mL/min.

For mass spectral analysis of the toluene extracts, the work was performed via two GC-MS instruments. The first one used the same temperature program and column stationary phase (0.25 mm x 30 m) as the above GC analysis on a Hewlett Packard 5971A mass selective detector (MSD), and MS Chem Station. The MSD was operated in electron impact (EI) mode with an ionization potential of 70 eV and a source temperature of 190°C. The scan range was 50-500 amu. The second GC-MS instrument was a HP 5210 Series 2 Plus, coupled to a Jeol GC Mate. The dual columns used were HP-5MS (0.25 mm x 30 m), with a temperature programming isothermally at 50°C for 2 min, then at 40°C/min to 200°C, and at 10°C/min to 300°C.

For HPLC analysis, a Hitachi L7000 system equipped with a Develosil ODS UG-5 column (2 x 150 mm) and photodiode array detector (225 nm) was used. The mobile phases were 10 mM ammonium acetate (pH 4.6) and MeCN, with a linear gradient increase of MeCN concentration from 30 to 100%. The flow rate was 0.2 mL/min and the volume of sample injected was 10 uL. For LC-MS analysis, a Hitachi M-1200H system equipped with Develosil ODS UG-5 column (4.6 x 150 mm) and photodiode array detector (225 nm) was used. The mobile phase consisted of 50% ammonium acetate and 50% MeCN. The flow rate was at 1.0 mL/min with the sample volume injected being 10 or 20 uL. The ionization was APCI (atomospheric pressure chemical ionization), with the aperture temperature at 130°C and the needle voltage at 3000 V. The scan range was 10-500 amu.

Interactions between heavy metals and Irgarol 1051 were also studied spectrophotometrically using a Shimadzu model UV-260 double beam UV-VIS recording spectrophotometer. An absorbance spectrum was typically taken between 190 and 400 nm.

RESULTS AND DISCUSSION

To exclude as much as possible the interference among chemicals in the reaction mixture, distilled water was used as the medium in which the effect of pH on the stability of Irgarol 1051 was investigated. Fresh distilled water was adjusted to pH values of 5.0, 7.0 and 9.0 with diluted acid (0.05 N HCl) and/or base (0.05 N NaOH) prior to introducing Irgarol 1051 to the test solutions. Figure 1 shows that Irgarol 1051 was very stable in distilled water at the acidic, neutral and alkaline pH tested (5.0, 7.0, 9.0). No significant change in Irgarol concentrations was observed in all the reaction mixtures during the 48-h test period, implying its inherent stability in aqueous solution. Since the pH for most natural waters ranges from 5 to 9 (Miyamoto et al., 1990), the continuous use of Irgarol 1051 as an antifouling agent may eventually result in its accumulation in the natural aquatic environment. Irgarol 1051 has been found to be highly stable in the marine environment and has been detected in areas close to intense boating activity (Scarlett et al., 1997).

Mercuric chloride is a microbial inhibitor, commonly used in the control of biodegradation studies to differentiate a chemical reaction from a microbiologically mediated process. During the early stage of our study on microbial degradation of Irgarol 1051, substantial amounts of breakdown products from Irgarol 1051 were found in the control flasks containing mercuric chloride. This led to the replacement of $HgCl_2$ by KCN (potassium cyanide) as an inhibitor in all subsequent experiments on microbial transformation of Irgarol 1051 (Liu et al., 1997). The process and mechanism involved in this chemical degradation of Irgarol 1051 by mercuric chloride were further investigated in the present study. Figure 1 clearly shows that, in the presence of a low concentration of $HgCl_2$ (20 mg/L), Irgarol 1051 would undergo a rapid chemical degradation in distilled water. Ten minutes after the initiation of the experiment, more than 90% of the added Irgarol had disappeared from the reaction mixture. The very fast disappearance of Irgarol 1051 strongly suggests that the degradation of Irgarol 1051 in distilled water by $HgCl_2$ closely follows the reaction of a catalyzed chemical hydrolysis. Reactions of a catalyzed hydrolysis are typically very fast to finish (Gamble and Khan, 1988), as was the case in the present investigation.

The $HgCl_2$-catalyzed hydrolysis of Irgarol 1051 in distilled water appears to be pH-independent (Figure 1). Virtually all the Irgarol 1051 had disappeared from the acidic, neutral, and alkaline distilled water (pH 5.0, 7.0 and 9.0) in less than 2 hours. The enhanced hydrolysis of Irgarol 1051 by $HgCl_2$ was also confirmed by another experiment, in which the appearance of the Irgarol's hydrolysis product M1 (2-methylthio-4-tert-butylamino-6-amino-s-triazine) in acidic, neutral and alkaline distilled water was followed (Figure 2). It can be seen that the formation of M1 was also not influenced by the pH of the distilled water used. M1 is a major metabolite resulting from the biotransformation of Irgarol 1051 by the white rot fungus Phanerochaete chrysosporium (Liu et al., 1997). Results from this study and our previous investigation (Liu et al., 1997) strongly suggest that M1 could be a major and perhaps ultimate degradation product during the chemical and biological degradation of Irgarol 1051. The observation of M1 formation from Irgarol 1051 via chemical or biological processes is of interest, particularly in view of the fact that hydroxyatrazine, an atrazine metabolite, could also be formed from atrazine by either chemical or biological reactions (Mandelbaum et al., 1993). Both atrazine and Irgarol 1051 are s-triazine herbicides and thus they likely share this similar property.

GC-MS confirmation of M1 as a hydrolytical product in the reaction mixture is shown in Figure 3. Figure 3a shows the total ion chromatogram of the DCM extract from an initial 10-min sample taken from the neutral distilled water containing 5 mg/L Irgarol 1051 and 10 mg/L $HgCl_2$. The chromatogram indicates the presence of two major compounds in the extract, Irgarol 1051 and the suspected hydrolysis product M1, which eluted from the column approximately 1.3 min earlier than the parent compound Irgarol. Figure 3c shows the (EI)GC-MS spectrum for Irgarol 1051. The mass spectrum is characterized by a molecular ion at m/z 253 and major fragment ions at m/z 238, 196, 182 and 112. These fragment ions can be attributed to fragmentation and/or rearrangements in the molecular ion and fragment ions (Liu et al., 1997). Figure 3b shows the mass spectrum of the suspected new hydrolysis product M1 formed during the $HgCl_2$-catalyzed hydrolysis of Irgarol 1051 in neutral distilled water. It can be seen that M1 has a molecular ion at m/z 213 and with major fragment ions at m/z 198, 157, and 111. The mass spectrum profile of this M1 mirrors exactly the one isolated during the fungal biotransformation of Irgarol 1051 (Liu et al., 1997). Thus, the M1 in the DCM extract of the $HgCl_2$-catalyzed hydrolysis of Irgarol 1051 in the neutral distilled water is very likely to be the compound 2-methylthio-4-tert-butylamino-6-amino-s-triazine.

Many triazines and their metabolites are thermally labile and cannot be always analyzed directly or reliably by GC or GC-MS. LC-MS and HPLC-MS offer alternatives for the direct analysis of triazine herbicides and their degradation products (Voyksner et al., 1987). In general, many of these degradation products have a polar structure, and thus can only be handled by GC or GC-MS after derivatization (Schroder, 1997). Figure 4 demonstrates the application of LC-MS in the identification of Irgarol 1051 and its degradation products. The LC profile of the DCM extract from a reaction mixture containing Irgarol 1051 and $HgCl_2$ is shown in Figure 4a. The high background noise is mainly due to salt (ammonium acetate) in the mobile phase. The peak at 11.32 min is the parent compound Irgarol 1051 and its APCI (atmospheric pressure chemical ionization) mass spectrum is shown in Figure 4d. The spectrum is very simple showing only two major ions, m/z 254 and 214, the former (m/z 254) being the protonated molecular ion $[M + H^+]$ of Irgarol 1051 and the latter (m/z 214) being the result of the loss of a cyclopropyl group from the protonated molecular ion m/z 254. APCI-MS employs soft ionization and thus the mass spectra acquired by

this technique are very simple, mainly consisting of only [M + H^+] ions (Voyksner et al., 1987). The peak at 4.03 min in the LC profile (Figure 4a) is the degradation product M1 resulting from the $HgCl_2$-catalyzed hydrolysis of Irgarol 1051. Its APCI-MS spectrum is shown in Figure 4b. The spectrum exhibits only two major ions, the m/z 214 being the protonated M1 molecular ion and the m/z 158 being the result of the loss of a tert-butyl group from the molecular ion m/z 214. The peak at 6.95 min in the LC profile in Figure 4a is probably the M1 monopotassium adduct, an artifact formed under the conditions of LC-APCI/MS analysis. Its mass spectrum is shown in Figure 4c. The ion m/z 252 is a M1 monopotassium adduct, i.e., [M1 + K^+] = (213 + 39 = 252). The ion m/z 214 is the protonated M1 molecular ion [M1 + H^+] = (213 + 1 = 214). The ions m/z 196 and 158 can be attributed to reactions involving adduct formation; [M1 + K^+ - isobutylene] = (213 + 39 - 56 = 196) and [M1 + K^+ + H^+ - K^+ - isobutylene] = (213 + 39 + 1 - 39 - 56 = 158). During ionization of pesticides by APCI, particularly those pesticides containing nitrogen (e.g., atrazine, metolachlor etc.), molecular and molecular adduct ions are formed preferentially over the fragmented ions (Schroder, 1997). Both Irgarol 1051 and its degradation product M1 contain nitrogen in their molecular structures, and thus their APCI-MS spectra exhibit molecular and adduct ions accordingly (Figure 4).

The reaction of mercury-catalyzed hydrolysis of Irgarol 1051 was also studied in buffer solutions at pH 5.0, 7.0 and 9.0 for an extended period of 120 hours to assess the influence of pH on the reaction of such a catalyzed hydrolysis (Figure 5). Formation of precipitate was immediately observed when mercuric chloride was added to the pH 9.0 buffer solution. The depletion of mercuric ions in the pH 9.0 buffer solution greatly increased the stability of Irgarol 1051 in that buffer solution. After a 24-h contact time, all Irgarol in the pH 5.0 and 7.0 buffer solutions had been completely hydrolyzed to yield the corresponding hydrolysis product M1. Only an insignificant amount of M1 was found in the pH 9.0 buffer solution after a 120-h contact time (Figure 5). The critical role of mercuric ions involved in enhanced hydrolysis of Irgarol 1051 was further demonstrated in another experiment, in which the addition of EDTA (50 mg/L) to the reaction mixture containing $HgCl_2$ (10 mg/L) and Irgarol 1051 (5mg/L) was found to be capable of suppressing the hydrolysis of Irgarol 1051 by approximately 50%. The amount of M1 produced was found to be only about half of what was produced from the reaction mixture containing no EDTA.

Direct evidence of the ability of mercuric chloride to react with Irgarol 1051 came from the UV (ultra violet) spectra (Figure 6) of the reaction mixtures containing a fixed amount (7 mg/L) of Irgarol 1051 and various amounts (0 - 22 mg/L) of $HgCl_2$. This evidence was further supported by the gas chromatographic determination of Irgarol 1051 and its hydrolysis product M1 in the reaction mixtures (Figure 7). It should be noted here that UV analysis for organics is less sensitive than that by GC, and thus the amount of $HgCl_2$ used in the reaction mixtures for the experiment of UV spectral analysis was doubled in comparison with the experiment for GC analysis. The results of Figure 6 indicate that a minute quantity of $HgCl_2$ (0.22 mg/L) was able to shift the absorbance maximum for Irgarol 1051 from 226.4 nm to 225.6 nm. A significant 8.8-nm down shift in absorbance maximum from 226.4 to 217.6 nm was observed when a higher concentration of $HgCl_2$ (21.84 mg/L) was used in the reaction mixture. Formation of the hydrolysis product M1 from Irgarol could be gas chromatographically demonstrated in the reaction mixture containing 0.1 mg/L $HgCl_2$. When the $HgCl_2$ was increased to 1 mg/L, approximately 50% of Irgarol 1051 in the reaction mixture had been hydrolyzed to M1. All the Irgarol 1051 had been hydrolyzed to M1 in

the presence of 10 mg/L $HgCl_2$.

The effectiveness of various heavy metal salts and one organomercury compound on the hydrolysis of Irgarol 1051 was also investigated in order to assess whether the observed mercuric chloride-catalyzed hydrolysis of Irgarol 1051 also occurs with other heavy metals (Table 1). Metal ions such as Cu(II) are known to accelerate the hydrolysis of some organic phosphate pesticides in a pH range between 5 and 6 where they are normally considered relatively stable (Mortland and Raman, 1967; Miyamoto et al., 1990). The results of Table 1 show that the four heavy metal salts $AgNO_3$, $CdCl_2$, $CuSO_4$, and $PbCl_2$ could not effect a hydrolysis of Irgarol 1051 in distilled water. No Irgarol disappearance and M1 formation in the reaction mixtures were observed. At the test concentration of 100 mg/L, $ZnCl_2$ was capable of catalyzing about 10% hydrolysis of Irgarol 1051. The ability of the inorganic mercury salt $HgCl_2$ to promote the hydrolysis of Irgarol 1051 was self-evident (Table 1). At the level of 10 mg/L, mercuric chloride was able to effect a complete hydrolysis of Irgarol 1051 in distilled water. However, the organic mercury compound MeHgCl was less effective in catalyzing the hydrolysis of Irgarol 1051. The cation Hg^{2+} appears to be responsible for the enhanced hydrolysis of Irgarol 1051, since the inclusion of EDTA (50 mg/L) in the reaction mixture would substantially lower the rate of Irgarol hydrolysis by $HgCl_2$.

Based on the limited information in the present study, a degradation pathway for the mercuric chloride-catalyzed hydrolysis of Irgarol 1051 is proposed (Figure 8). The pathway includes the initiation of the reaction by mercuric ion to form a four-membered chelate complex involving the Irgarol ring nitrogen (number 5 position) and the nitrogen at the 6-cyclopropylamino side chain. This interaction increases the electrophilic character of the cyclopropyl carbon adjacent to the amino nitrogen of the cyclopropylamino side chain and would simultaneously weaken the cyclopropyl-NH bond, thus facilitating the cleavage of this bond by the nucleophilic attack of water. This reaction would lead to the formation of two degradation products M1 and cyclopropanol. Being more stable than the parent compound, M1 has been frequently identified in the reaction mixtures. However, our attempts to identify cyclopropanol as a degradation product were not successful. Cyclopropanol is an extremely unstable compound, primarily due to its angle strain. Many attempts to prepare cyclopropanol resulted only in the formation of allyl alcohol (Fieser and Fieser, 1964).

Although there are several reports delineating the hydrolysis of pesticides by metal ions (Miyamoto et al., 1990; Munch and Frebis, 1992; Noblet et al., 1996), the mechanism of metal ion-catalyzed hydrolysis of pesticides in the aquatic environment is poorly understood. Mortland and Raman (1967) studied the Cu(II)-catalyzed hydrolysis of organophosphate pesticides, and proposed an elegant chemical degradation mechanism. The proposed mechanism involved the bidentating chelation of the metal ion Cu(II) through nitrogen in the ring structure and sulfur on the phosphate side chain in the studied pesticides Dursban and Diazinon. The resultant cyclic resonance system would weaken the bonding of the side chain through electron shifts, thus promoting the hydrolysis. Pusino et al. (1988) also proposed a similar degradation mechanism involving the bidentate complex to explain the catalytic hydrolysis of the pesticide Quinaphos by Cu(II), Fe(III) and other metal ions. Hydrolysis is an important abiotic degradation process for many pesticides in aquatic environments. Thus, understanding the factors affecting the rate of hydrolysis is essential in estimating a pesticide's environmental persistence. Although the studies presented here deal with a pure herbicide tested in the laboratory setting, and although it may be difficult to extrapolate the

results to the natural environment, the data identify a significant deficiency in information regarding the impact of heavy metals on the environmental persistence of pesticides. Heavy metal and pesticide contaminants can be expected to be found together in the environment and in order to more accurately estimate the potential impact of these compounds, further interaction studies must be undertaken. For this reason, abiotic degradation of priority chemicals is also one of the major and long term studies being conducted in our laboratories. Probably one of the most important and practical aspects of this work is the demonstration of rapid decompostion of Irgarol 1051 by mercuric chloride. Consequently, $Hgcl_2$ should never be used as a microbial inhibitor in preserving water samples in Irgarol monitoring programs. Mercuric chloride has been widely as microbial inhibitor to control biological degradation in environmental samples including the US EPA National Pesticide Survey Program (Munch and Frebls, 1992).

CONCLUSIONS

Specific conclusions established from this study are as follows.

1. Mercuric chloride was capable of rapidly catalyzing the hydrolysis of the new antifouling compound Irgarol 1051 in distilled water and buffer solutions.

2. The abiotic degradation appeared to follow the reaction of a catalyzed hydrolysis, and was not significantly affected by the pH tested (5 to 9).

3. All other 5 heavy metal salts tested (($AgNO_3$, $CdCl_2$, $CuSO_4$, $PbCl_2$, and $ZnCl_2$) had practically no catalytic property on Irgarol hydrolysis, implying the involvement of a specific activity for Hg^{2+} in this reaction.

4. The mechanism for the catalyzed hydrolysis may be the formation of bidentate chelation through nitrogen (No. 5) on the ring and the nitrogen on the cyclopropylamino side chain in Irgarol 1051 with the Hg^{2+} ion. The resulting four-member chelate complex would weaken the cyclopropyl-amino bond considerably, thus facilitating the hydrolysis reaction.

5. Ultraviolet spectroscopy of the reaction mixtures and the identification of Irgarol hydrolysis product M1 (2-methylthio-4-tert-butylamino-6-amino-s-triazine) by GC-MS and LC-MS provided the basis for the proposed mechanism on the $HgCl_2$-catalyzed hydrolysis of Irgarol 1051. M1 appeared to be more stable than the parent compound Irgarol 1051, thus implying its possible accumulation in the environment.

6. One practical aspect of this work is that $HgCl_2$ should not be used in preserving water samples in Irgarol 1051 monitoring programs.

ACKNOWLEDGEMENTS

This work was supported in part by (1.) the PESTMYOP and Great Lakes 2000 funds of the Department of Environment (Canada), and by (2.) a research grant provided by the Ministry of Education, Science, Sports and Culture (Japan) for the International Comparative Study on Toxicity Assessment. The authors would also like to thank Drs. C.E. Cerniglia and T.M. Heinze of the National Center for Toxicological Research, Jefferson, for the interpretation of some of the mass spectral data.

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LEGENDS

Figure 1. Time course of the Irgarol's concentration profiles in the presence and absence of mercuric chloride.

Figure 2. Formation of the Irgarol's degradation product M1 in the presence and absence of mercuric chloride.

Figure 3. Chromatogram and mass spectra: (a) total ion chromatogram of the DCM extract from an initial 10-min sample taken from the neutral distilled water containing 5 mg/L Irgarol 1051 and 10 mg/L $HgCl_2$, (b) low-resolution mass spectrum of M1 from the peak M1 in Fig. 3(a), and (c) low-resolution mass spectrum of Irgarol from the Irgarol peak in Fig.3(a).

Figure 4. Chromatogram and mass spectra: (a) LC chromatogram of the DCM extract from a reaction mixture containing Irgarol 1051 and $HgCl_2$, (b) mass spectrum of M1 from the 4.03 min peak in Fig. 4(a), (c) mass spectrum of M1 monopotassium adduct from the 6.95 min peak in Fig. 4(a), and (d) mass spectrum of Irgarol 1051 from the 11.32 min peak in Fig. 4(a).

Figure 5. Time course of Irgarol 1051 and M1 concentration profiles in buffer solutions containing 5 mg/L Irgarol and 20 mg/L $HgCl_2$ at pH 5.0. 7.0 and 9.0.

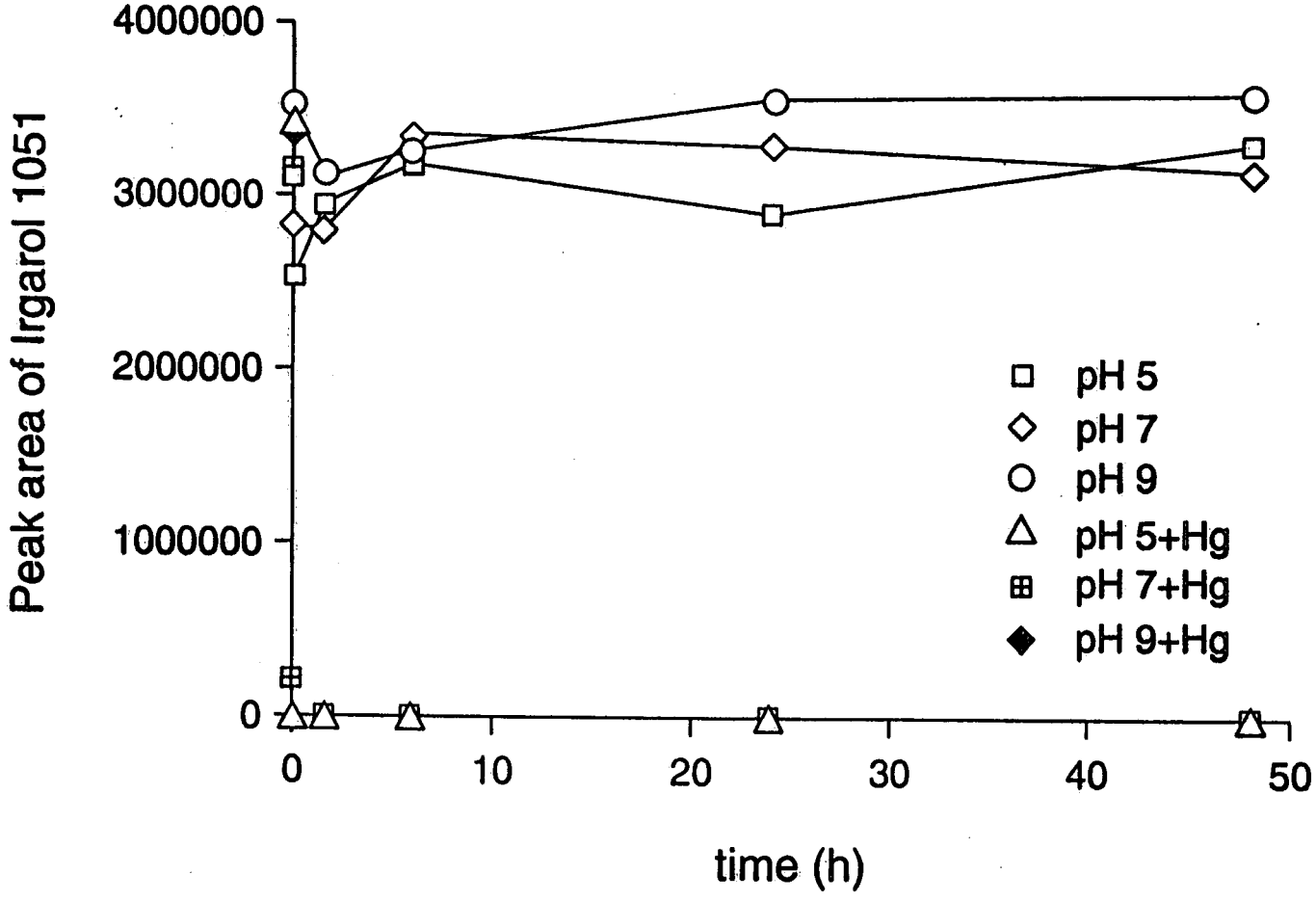
Figure 6. Effect of $HgCl_2$ on the UV absorbance spectra of Irgarol 1051. The reactants were incubated at room temperature (21°C) for 1 hour before spectra taken.

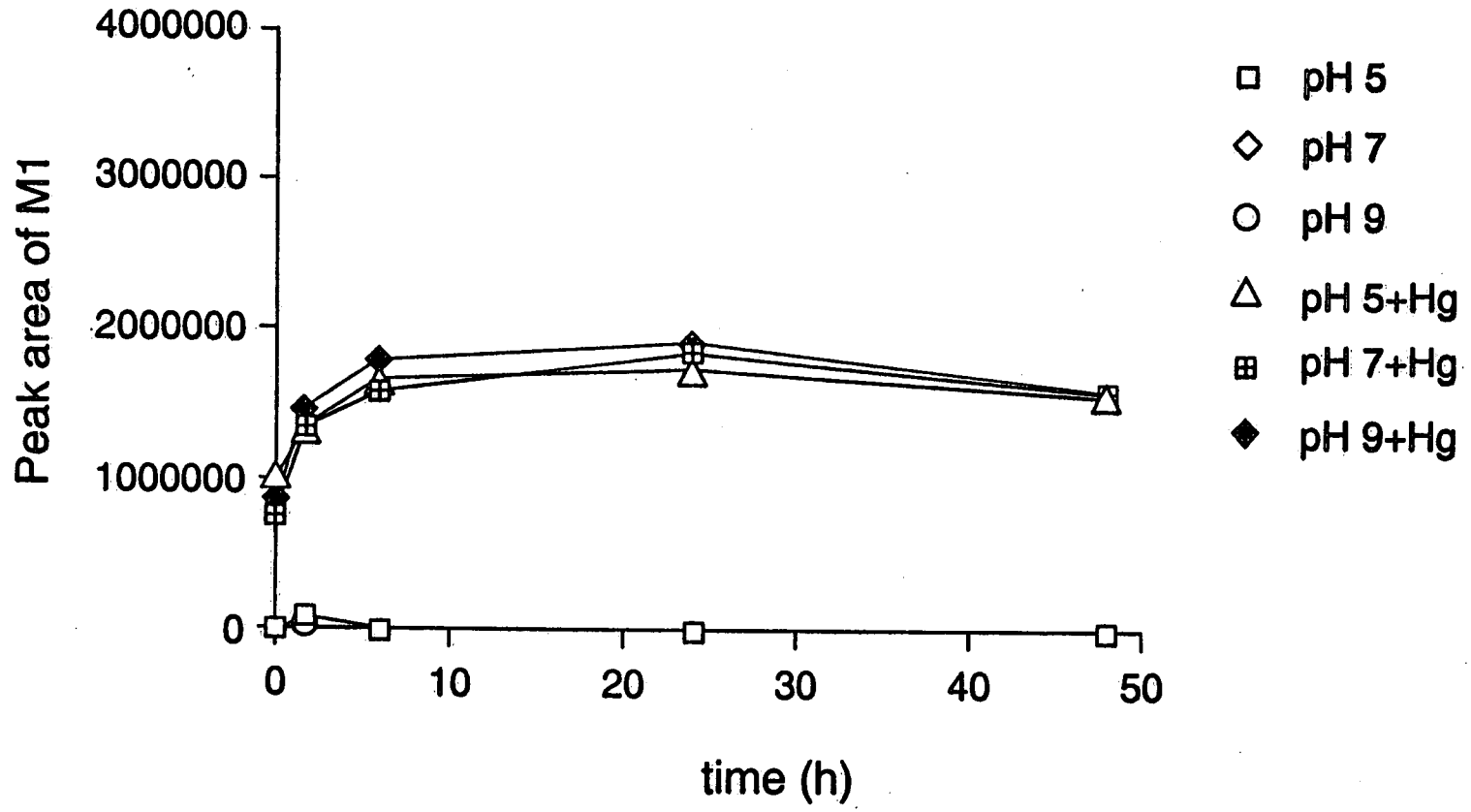
Figure 7. Concentration effect of $HgCl_2$ on Irgarol degradation and M1 formation. The reactants were incubated at room temperature (21°C) for 1 hour before extraction with DCM for gc analysis.

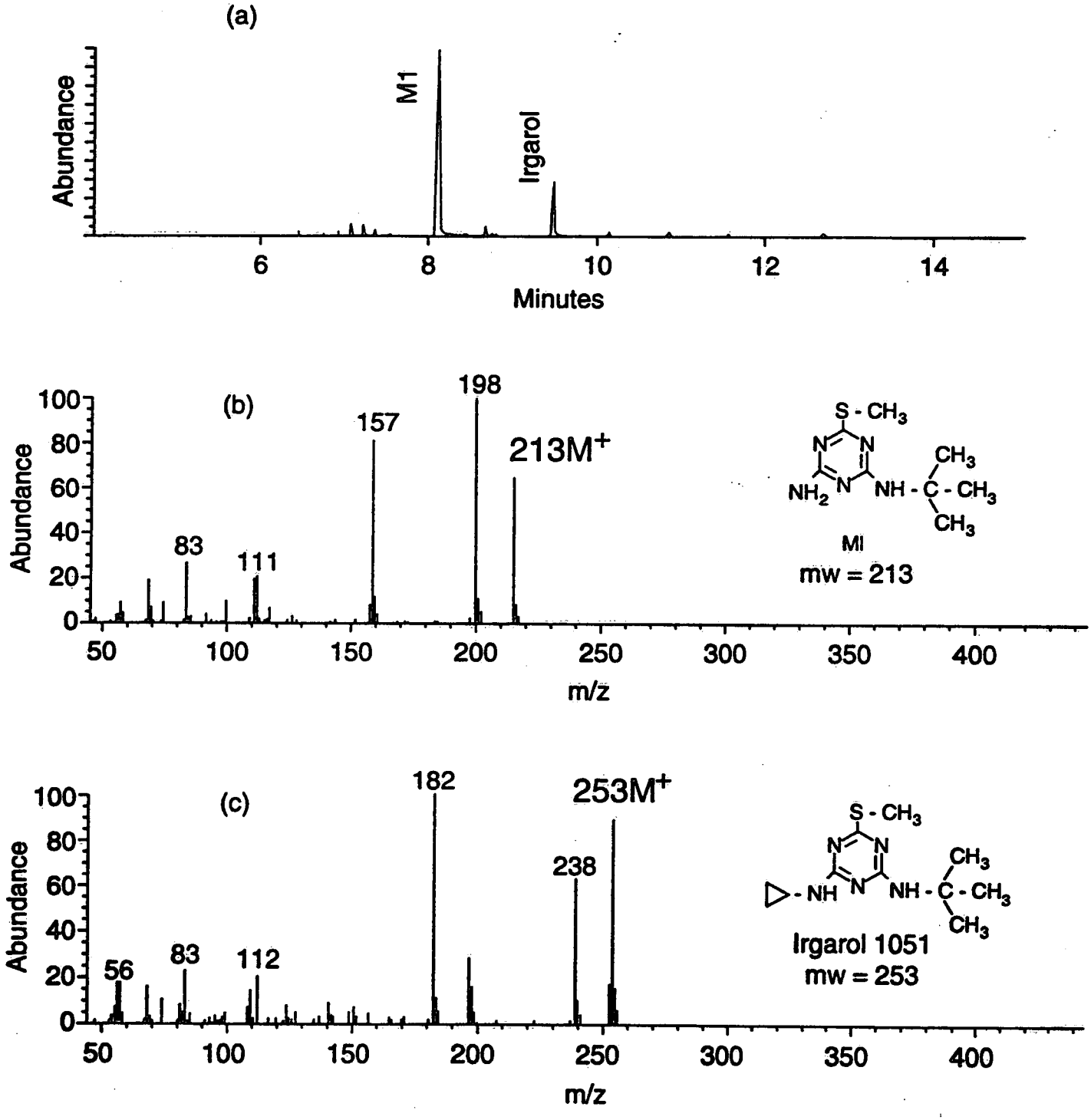
Figure 8. Proposed mechanism for the $HgCl_2$-catalyzed hydrolysis of Irgarol 1051.

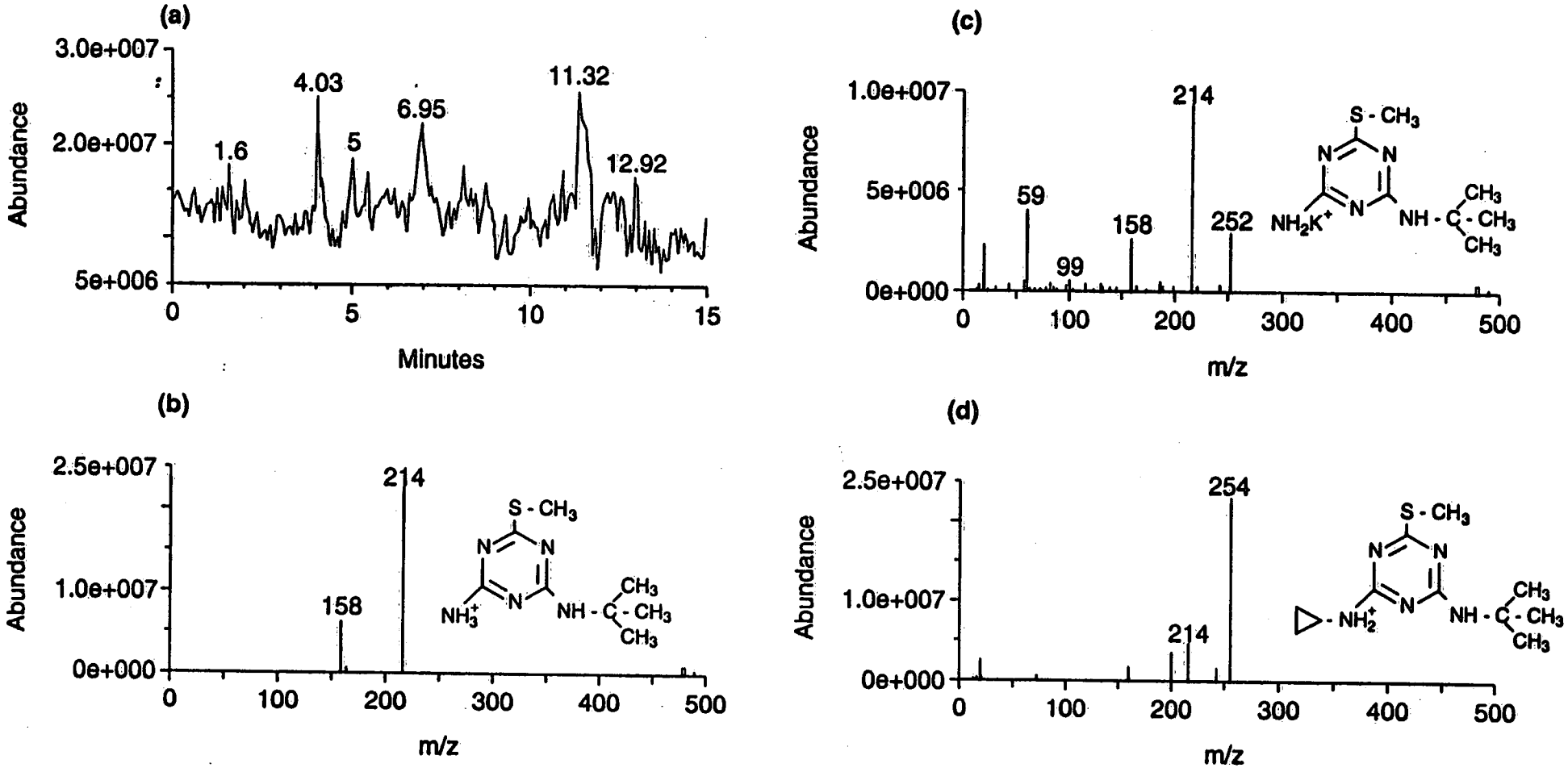
Table 1. Hydrolysis of Irgarol 1051 by heavy metals

Metals	% Irgarol hydrolyzed		
	10 mg/L	20 mg/L	100 mg/L
$CuCl_2$	0	0	0
$AgNO_3$	0	0	0
$CdCl_2$	0	0	0
$PbCl_2$	0	0	0
$ZnCl_2$	0	0	10
$HgCl_2$	100	100	100
MeHgCl	13	33	62


Peak area of Irgarol 1051
4000000
3000000
2000000
1000000
0
0
10
20
30
40
50
time (h)
pH 5
pH 7
pH 9
pH 5+Hg
pH 7+Hg
pH 9+Hg


4000000
3000000
2000000
1000000
0
Peak area of M1
0
10
20
30
40
50
time (h)
pH 5
pH 7
pH 9
pH 5+Hg
pH 7+Hg
pH 9+Hg


(a)
Abundance
M1
Irgarol
6
8
10
12
14
Minutes
(b)
100
80
60
40
20
0
Abundance
198
157
213M+
83
111
50
100
150
200
250
300
350
400
m/z
S - CH3
N
N
NH2
N
NH - C - CH3
CH3
CH3
MI
mw = 213
(c)
182
253M+
238
56
83
112
m/z
Irgarol 1051
mw = 253


(a)
3.0e+007
2.0e+007
5e+006
Abundance
1.6
4.03
5
6.95
11.32
12.92
0
5
10
15
Minutes
(b)
2.5e+007
1.0e+007
0e+000
Abundance
158
214
S - CH3
N
N
NH3+
N
NH - C - CH3
CH3
CH3
0
100
200
300
400
500
m/z
(c)
1.0e+007
5e+006
0e+000
Abundance
59
99
158
214
252
S - CH3
N
N
NH2K+
N
NH - C - CH3
CH3
CH3
0
100
200
300
400
500
m/z
(d)
2.5e+007
1.0e+007
0e+000
Abundance
214
254
S - CH3
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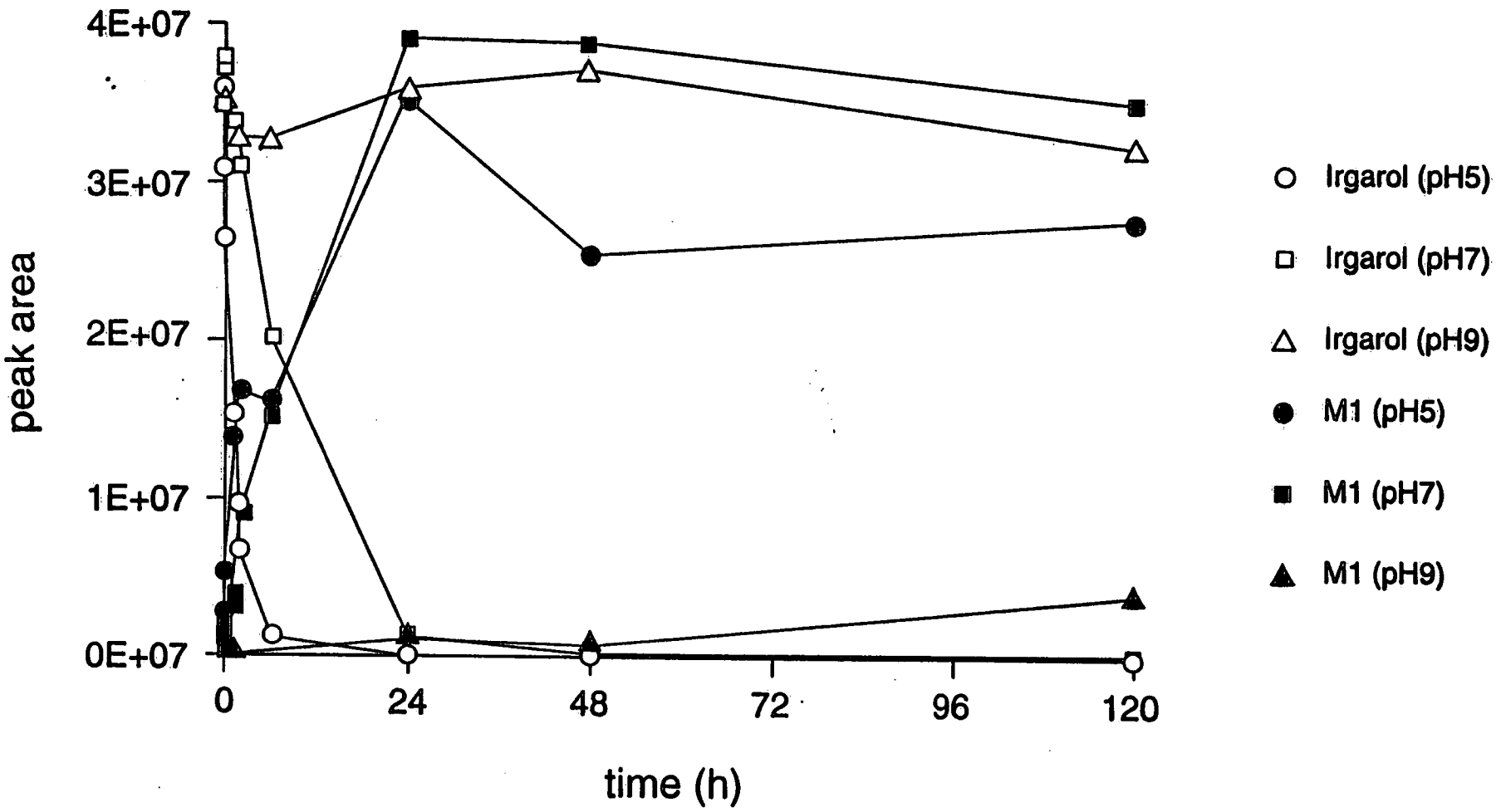
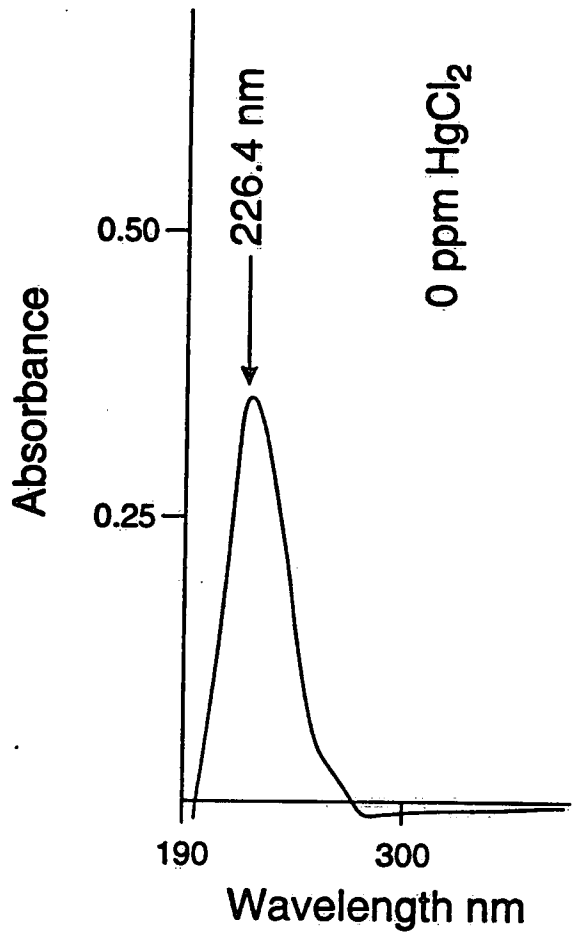
N
NH2+
N
NH - C - CH3
CH3
CH3
0
100
200
300
400
500
m/z


4E+07
3E+07
2E+07
1E+07
0E+07
peak area
0
24
48
72
96
120
time (h)
Irgarol (pH5)
Irgarol (pH7)
Irgarol (pH9)
M1 (pH5)
M1 (pH7)
M1 (pH9)


Absorbance
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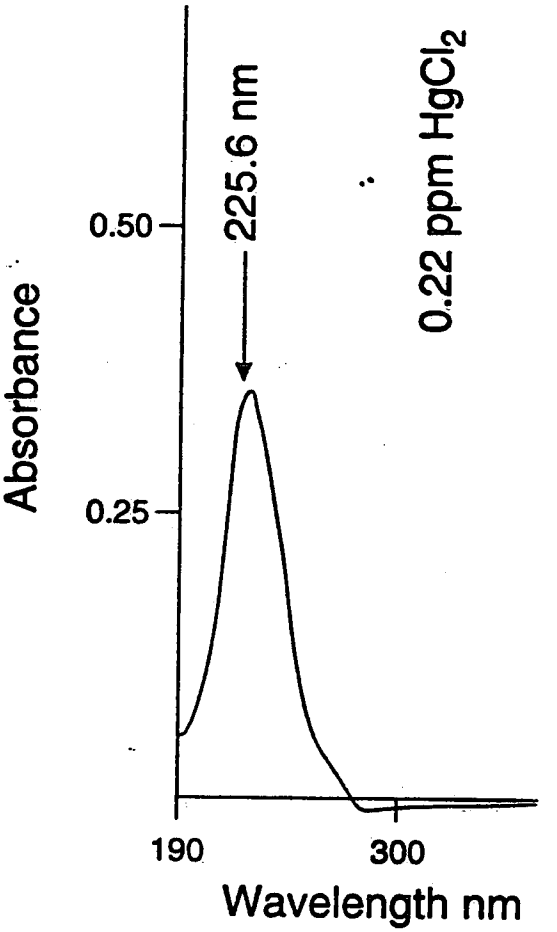
0.25
226.4 nm
0 ppm $HgCl_2$
190
300
Wavelength nm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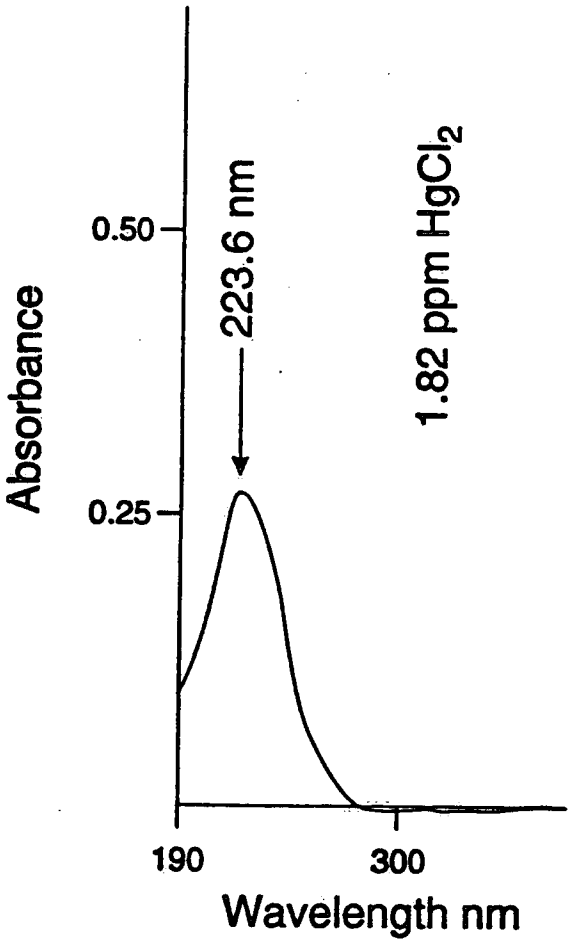
225.6 nm
0.22 ppm $HgCl_2$
190
300
Wavelength nm


Absorbance
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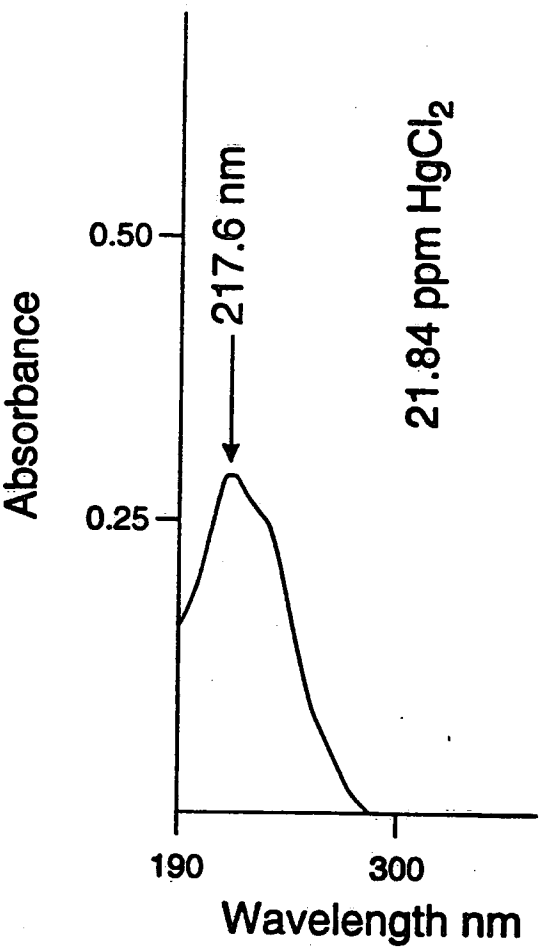
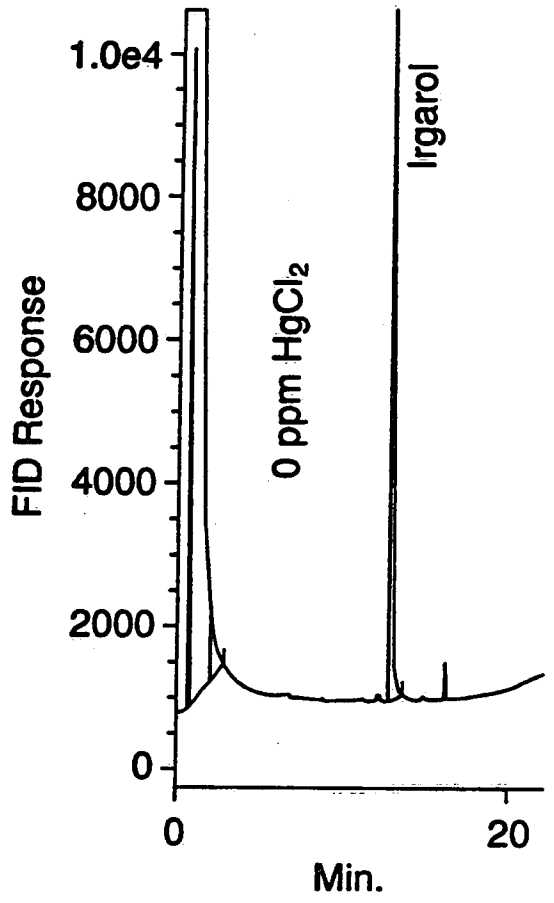
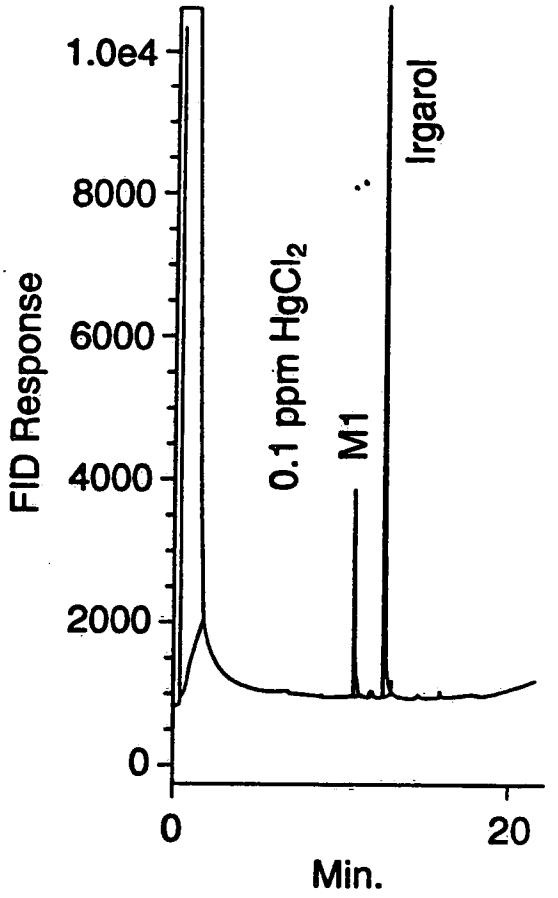
0.25
223.6 nm
1.82 ppm $HgCl_2$
190
300
Wavelength nm


Absorbance
0.50
0.25
217.6 nm
21.84 ppm $HgCl_2$
190
300
Wavelength nm


1.0e4
8000
6000
4000
2000
0
FID Response
0 ppm $HgCl_2$
Irgarol
0
20
Min.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8000
6000
4000
2000
0
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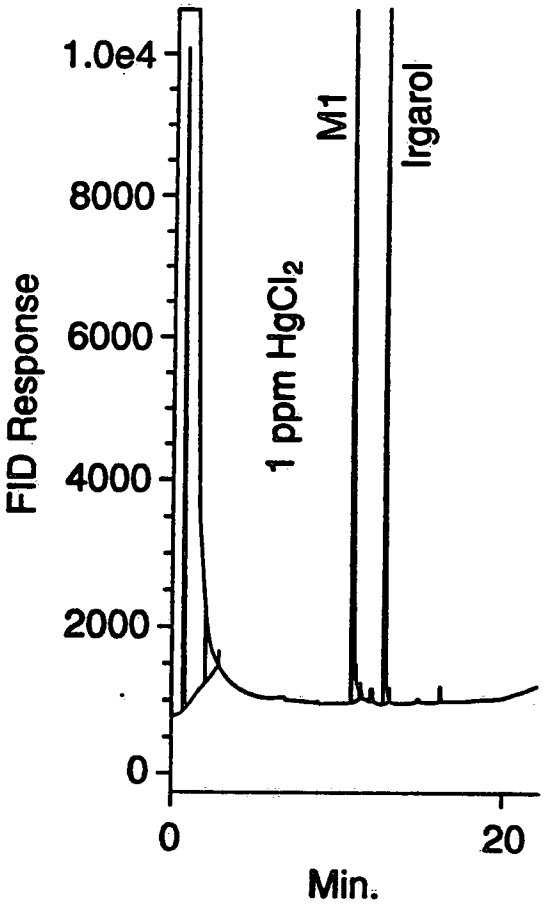
0.1 ppm $HgCl_2$
M1
Irgarol
0
20
Min.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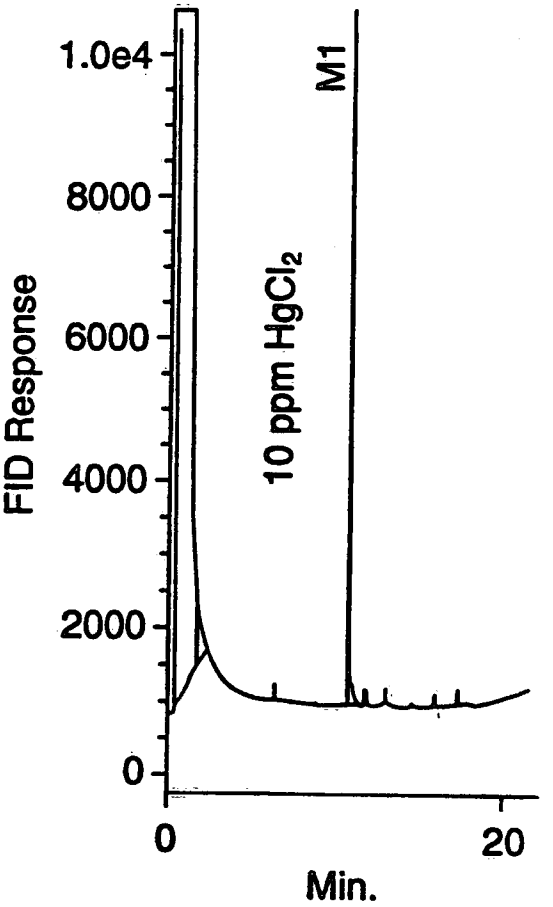
8000
6000
4000
2000
0
FID Response
1 ppm $HgCl_2$
M1
Irgarol
0
20
Min.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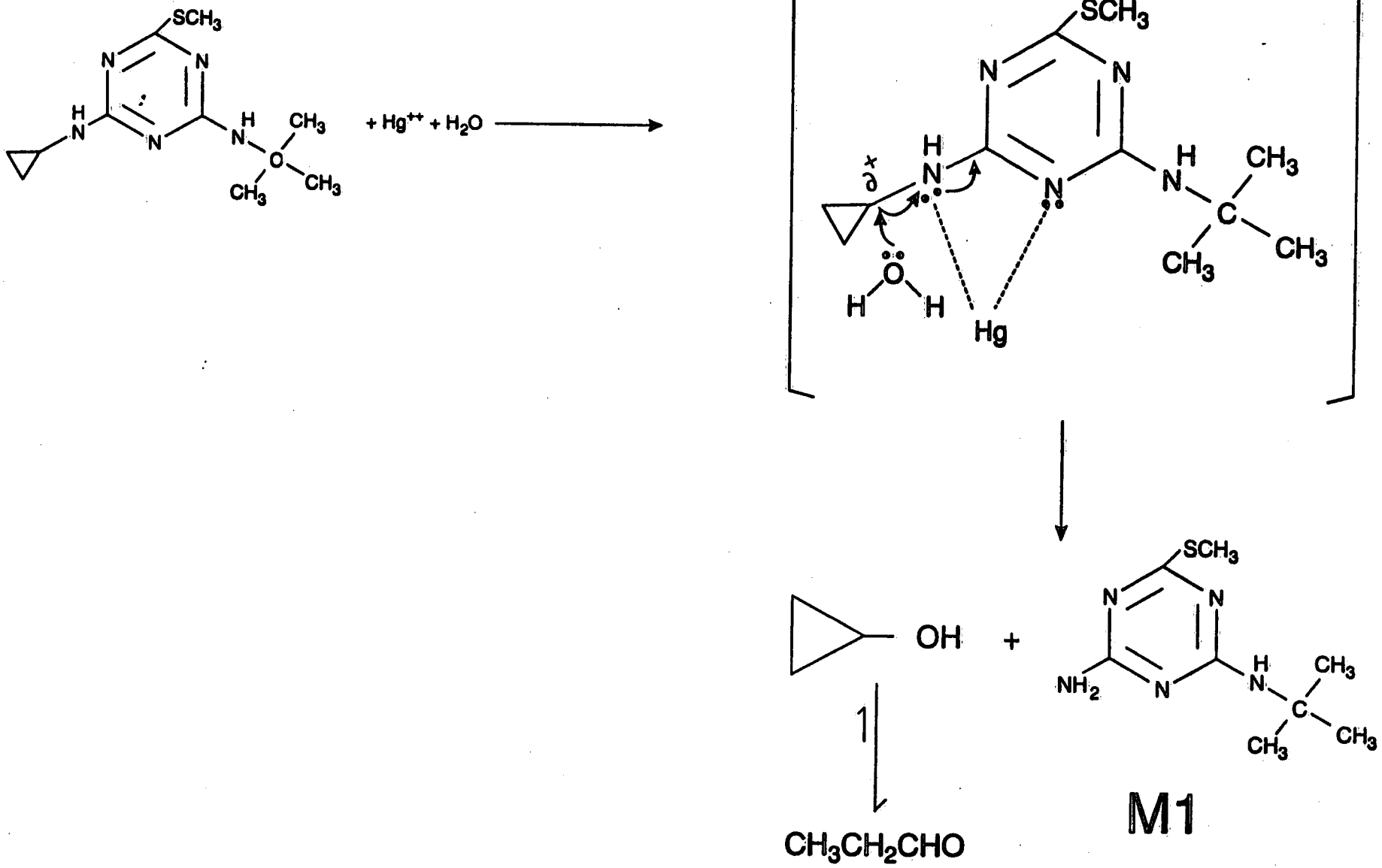
8000
6000
4000
2000
0
FID Response
10 ppm $HgCl_2$
M1
0
20
Min.


SCH3
N
N
H
N
N
H
N
CH3
O
CH3
CH3
+ Hg++ + H2O
SCH3
N
N
H
N
N
H
N
CH3
C
CH3
CH3
δ+
O
H
H
Hg
OH
+
SCH3
N
N
NH2
N
H
N
CH3
C
CH3
CH3
1
CH3CH2CHO
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