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NWRI CONTRIBUTION NO. 96-171

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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 the copper-based antifouling paints. It is intended to replace the widely used antifouling agent tributyltin (TBT) which has been banned internationally since 1990, primarily due to its severe impact on the aquatic ecosystem. For example, it has been estimated 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, or the so-called imposex problem), and thus seriously reducing the population of gastropods in the marine environment.

The use of a suitable herbicide such as Irgarol 1051 in the copper-based antifouling paints is necessary, because the copper salts themselves are not effective in inhibiting the primary colonization of a ship's hull surface by micro-algae and subsequent growth of seaweeds. Fouling on ships causes hull roughness leading to increased frictional resistance and an increase in 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 for which there is no information in the open literature on the persistence and degradation: a fact that hinders the assessment of its ultimate impact on the environment. This study shows that the white rot fungus *Phanerochaete chrysosporium* was capable of biotransforming Irgarol 1051 via partial N-dealkylation. Metabolic dealkylation occurs at the cyclopropylamino group resulting in metabolite M1 (2-methylthio-4-*tert*-butylamino-6-amino-*s*-triazine). No evidence of the heterocyclic ring cleavage of Irgarol 1051 was observed, thus implying a possibility of its degradation products accumulation in the environment. This is the first report of metabolites of Irgarol 1051.

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, we are presently determining whether it is present in several large harbours in Canada, from the leaching of ships painted in other countries.

It may be appropriate to note here the rationale for using the white rot fungus *Phanerochaete chrysosporium* to study the biotransformation of the new antifouling compound Irgarol 1051. *Phanerochaete chrysosporium* has been shown to possess biodegradative capabilities for a broad spectrum of environmentally persistent contaminants, including DDT, PCBs, and pentachlorophenol. In addition, it is also the most potent and best-characterized lignin-degrading microorganisms available. Therefore, the employment of *Phanerochaete chrysosporium* in our Irgarol study would ensure a greater chance of success. This report confirms the soundness of our research strategy.

SOMMAIRE À L'INTENTION DE LA DIRECTION

L'Irgarol 1051, soit la 2-méthylthio-4-*tert*-butylamino-6-cyclopropylamino-*s*-triazine, est un additif herbicide nouvellement mis au point, destiné à être utilisé dans les peintures antisalissures à base de cuivre. Ce produit est destiné à remplacer l'agent antisalissure tributyl- étain (TBT) dont l'utilisation est interdite depuis 1990 à l'échelle internationale, en raison de son impact important sur l'écosystème aquatique. Par exemple, l'utilisation de TBT uniquement dans le bassin d'Arcachon, en France, a causé, estime-t-on, des pertes de revenu de 147 millions de dollars américains dans l'industrie ostréicole. Des études très récentes ont montré que le TBT peut provoquer des changements sexuels chez les gastropodes. Des caractéristiques mâles (par exemple, pénis ou problème couramment appelé «imposexe») apparaissaient chez des femelles qui avaient été exposées à des concentrations de l'ordre de parties par billion (ppt), ce qui entraînait une grave réduction de la population de gastropodes dans l'environnement marin.

Il faut utiliser un herbicide convenable, tel que l'Irgarol 1051, dans les peintures antisalissures à base de cuivre, car les sels de cuivre ne peuvent pas, à eux seuls, inhiber efficacement la colonisation primaire de la coque d'un navire par des microalgues et la croissance ultérieure d'algues marines. Les salissures rendent la coque des navires rugueuse, ce qui se traduit par une résistance de frottement accrue et une plus grande consommation de carburant. La présence d'une couche de salissures d'une épaisseur de 1 mm sur la coque d'un navire entraînerait, estime-t-on, une augmentation de 80 % du frottement et une perte de vitesse de 15 %. L'application d'une peinture antisalissure sur les bateaux a donc des conséquences économiques très importantes. L'Irgarol est un nouveau produit chimique pour lequel on ne trouve aucune donnée sur la persistance et la dégradation dans la documentation accessible à tous, ce qui nuit à l'évaluation de son impact ultime sur l'environnement. Cette étude montre que le champignon de la pourriture blanche *Phanerochaete chrysosporium* peut biotransformer l'Irgarol 1051 par N-désalkylation partielle. La désalkylation métabolique intervient au groupe cyclopropylamino pour donner le métabolite M1 (2-méthylthio-4-*tert*-butylamino-6-amino-*s*-triazine). Rien n'indique qu'il y a clivage du noyau hétérocyclique de l'Irgarol 1051, ce qui laisse supposer qu'il y a accumulation possible de ses produits de dégradation dans l'environnement. C'est la première fois que l'on fait état de métabolites de l'Irgarol 1051.

Actuellement, l'Irgarol 1051 n'est pas homologué au Canada en vertu de la Loi sur les produits antiparasitaires. En prévision de son homologation et compte tenu du fait qu'il est homologué dans certains autres pays, nous cherchons actuellement à déterminer si les eaux de plusieurs grandes baies au Canada contiennent de l'Irgarol 1051qui proviendrait alors de bateaux peinturés dans d'autres pays.

Il y aurait peut-être lieu de préciser ici les raisons qui nous ont motivés à utiliser le champignon de la pourriture blanche *Phanerochaete chrysosporium* pour étudier la biotransformation du nouveau composé antisalissure Irgarol 1051. Le champignon *Phanerochaete chrysosporium* peut, on l'a montré, biodégrader une vaste gamme de contaminants qui persistent dans l'environnement, par exemple le DDT, les BPC et le pentachlorophénol. De plus, il est le plus puissant et le mieux caractérisé des micro-organismes assurant la dégradation de la lignine. L'utilisation du champignon *Phanerochaete chrysosporium* dans notre étude sur l'Irgarol améliorera donc nos chances de succès. Ce rapport vient confirmer le bien-fondé de notre stratégie de recherche.

ABSTRACT

Irgarol 1051, 2-methylthio-4-*tert*-butylamino-6-cyclopropylamino-*s*-triazine, is a newly developed herbicidal additive for use in the copper-based antifouling paints. It is intended to replace the antifouling agent tributyltin which has been banned internationally due to its severe impact on the aquatic ecosystem. There is, however, no information in the open literature on the persistence and degradation of Irgarol, a fact that hinders the assessment of its ultimate impact on the environment. This study showed that the white rot fungus *Phanerochaete chrysosporium* was capable of biotransforming Irgarol 1051. It appears that the metabolism of Irgarol in the fungus proceeds mainly via partial N-dealkylation. Metabolic dealkylation occurs at the cyclopropylamino group resulting in metabolite M1, which has tentatively been identified as 2-methylthio-4-*tert*-butylamino-6-amino-*s*-triazine. M1 appeared to be a stable and/or terminal metabolite. No evidence of the heterocyclic ring cleavage of Irgarol 1051 was observed, thus implying a possibility of its degradation products accumulating in the environment.

Key Words: Irgarol 1051, fouling, antifouling compound, herbicide, biotransformation, metabolite, metabolic pathway, fungus, N-dealkylation,
Phanerochaete chrysosporium

RÉSUMÉ

L'Irgarol 1051, soit la 2-méthylthio-4-*tert*-butylamino-6-cyclopropylamino-*s*-triazine, est un additif herbicide nouvellement mis au point, destiné à être utilisé dans les peintures antisalissures à base de cuivre. Ce produit est destiné à remplacer l'agent antisalissure tributyl- étain dont l'utilisation a été interdite à l'échelle internationale, en raison de son impact important sur l'écosystème aquatique. Toutefois, la documentation accessible à tous ne renferme aucune donnée sur la persistance et la dégradation de l'Irgarol, ce qui nuit à l'évaluation de son impact ultime sur l'environnement. Cette étude montre que le champignon de la pourriture blanche *Phanerochaete chrysosporium* peut biotransformer l'Irgarol 1051. Il semble que le métabolisme de l'Irgarol dans le champignon fait intervenir principalement une N-désalkylation partielle. La désalkylation métabolique intervient au groupe cyclopropylamino pour donner le métabolite M1 que l'on a identifié pour le moment comme la 2-méthylthio-4-*tert*-butylamino-6-amino-*s*-triazine. Le métabolite M1 semble être un métabolite stable et/ou terminal. Rien n'indique qu'il y a clivage du noyau hétérocyclique de l'Irgarol 1051, ce qui laisse supposer qu'il y a accumulation possible de ses produits de dégradation dans l'environnement.

Mots clés: Irgarol 1051, salissure, composé antisalissure, herbicide, biotransformation, métabolite, voie métabolique, champignon, N-désalkylation, *Phanerochaete chrysosporium*

INTRODUCTION

Objects submerged in the aquatic environment are subject to "fouling", which has been defined as the settlement and growth of micro- or marcro-organisms on any man-made or natural submerged substratum (Evans and Hoagland, 1986). Fouling can cause significant financial losses to industry. One notable example is shipping, where a growth of merely 100 μm on a ship's hull can cause a 6% increase in fuel consumption. In addition to the increased fuel cost, expensive antifouling procedures are also required. Industrial cooling towers and heat exchangers also have their efficiencies reduced by fouling because the growth inhibits heat transfer. Fouling increases flow resistance and reduces the flow carrying capacity of pipelines.

Numerous approaches in fouling control have been considered, but the application of biocide(s)-laden antifouling paint remains the most important and cost-effective measure (Bowden and Taylor, 1980). 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 prohibit 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). The use of a herbicide in copper-based antifouling paints is essential because the copper salts themselves are not particularly effective in inhibiting the primary colonization of the hull surface by micro-algae and the subsequent growth of seaweeds (Gough *et al.*, 1994).

Irgarol 1051, 2-methylthio-4-*tert*-butylamino-6-cyclopropylamino-*s*-triazine, is a newly developed herbicidal additive for use in the copper-based antifouling paints. Irgarol does not appear to undergo rapid biodegradation (Ciba-Geigy, 1995), and its residues have been detected in European coastal waters (Readman *et al.*, 1993; Gough *et al.*, 1994) and lake water (Toth *et al.*, 1996). To our best knowledge there is no information about its environmental fate and persistence. In this communication we report the biotransformation of Irgarol 1051 by the white rot fungus *Phanerochaete chrysosporium*, and the

tentative identification of a metabolite (2-methylthio-4-*tert*-butylamino-6-amino-*s*-triazine) during the biotransformation process.

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 the Ciba-Geigy Canada Ltd., Mississauga, Ontario L5M 5N3. For ease of reference, the molecular structure of Irgarol 1051 and its fungal metabolite M1 are illustrated in Figure 1. 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 was also rinsed with pesticide grade solvents before use in the experiments. All other chemicals used in the experiments were reagent grade or better.

Microorganisms and Culture Conditions

Resistance of Irgarol 1051 to fungal degradation was assessed using the white rot fungus *Phanerochaete chrysosporium* (ATCC 20696). The solid growth medium was a modified malt extract medium (MME) with the composition of 6.0 g of malt extract base, 1.8 g of maltose, 6.0 g of glucose, 120 mg of yeast extract and 15 g of agar per litre of distilled water. An appropriate amount of Irgarol 1051 (in methanol) was added directly to this growth medium to achieve a final Irgarol concentration of approximately 7 mg/L. The pH of the medium was then adjusted to 4.7 prior to its sterilization at 121°C for 15 min.

To determine the fungal transformation of Irgarol 1051 on solid growth medium, agar plates (MME) with or without Irgarol 1051 (7 mg/L) were inoculated with fungal plugs taken from a mature *Phanerochaete chrysosporium* plate, and were incubated together with control plates (i.e., agar plates without inoculation) at room temperature (21°C) for various time intervals. Sampling times for Irgarol

1051 and its metabolites were guided by the observation of the fungal growth rate radiating from the plug on the inoculated agar plates.

Sample Preparation and Chemical Analysis

To assess the fungal transformation of Irgarol 1051 by *Phanerochaete chrysosporium* the inoculated and control agar plates were sacrificed at appropriate time intervals. The agar in each plate was cut into small pieces (appr. 5 x 5 mm) with a stainless knife and placed in a 125-mL Erlenmeyer flask containing 50 mL of dichloromethane (DCM). The flask was placed on a rotary shaker (220 rpm) for 1 hr and the DCM was decanted from the flasks and dried through anhydrous sodium sulphate. A toluene keeper was added to the resulting extracts which were then concentrated to 5 mL on a rotary evaporator. Further concentration and solvent exchange into toluene were performed under a nitrogen stream.

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. Mass spectral analysis was performed using the same temperature program and column stationary phase (0.25 mm x 30 m) 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.

RESULTS AND DISCUSSION

Unlike bacterial metabolism of organic compounds, fungal biodegradation is often characterized by the accumulation of many degradation products including those derived from the metabolism of organic ingredients in the growth medium. The extensive accumulation of these normal growth metabolites sometimes may overshadow the formation of new metabolites from the test chemical, thus complicating

the identification of these new metabolites. Because of this potential complication, several controls were used, e.g., a growth control (fungal growth of MME medium without Irgarol 1051), a medium control (sterile MME medium), and a chemical control (sterile MME medium plus Irgarol 1051) in the fungal biotransformation experiments. The NPD chromatograms of the DCM extracts from a fungal growth control plate (i.e., MME plate without Irgarol) and an Irgarol degradation plate (i.e., MME plate with Irgarol) are shown in Figure 2. Figure 2A shows the production of normal growth metabolites from organic nutrients in the regular growth medium (MME) by a culture of *P. chrysosporium*. The profiles of the two chromatograms clearly demonstrate the advantages of using the fungal growth control in pinpointing the chromatographic peak M1 (Figure 2B) as a possible new metabolite during the biotransformation of the antifouling compound Irgarol 1051 by the white rot fungus *P. chrysosporium*.

Irgarol 1051 is a new chemical, and to our best knowledge there are only three publications on Irgarol (Readman *et al.*, 1993; Gough *et al.*, 1994; Toth *et al.*, 1996), all dealing with its environmental concentrations in the European coastal water and lake water. There has been no information on its persistence/degradation, a major criterion needed in the analysis of environmental impact of chemicals. Consequently, a computer search of the CAS mass spectra library for Irgarol 1051 was initiated, and again the search indicated no mass spectral data for this chemical. Without a reasonable size of literature data for comparison, the interpretation of our experimental results on Irgarol's degradation should be regarded as contingent. Figure 3 shows the (EI)GC-MSD spectrum for Irgarol 1051. The mass spectrum was 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. The $(M - 15)^+$ ion (m/z 238), most probably a 2-thio-4-*tert*-butylamino-6-cyclopropylamino-*s*-triazine, arose from the cleavage of the methyl group with the 2-methylthio group in the molecular ion (m/z 253). The $(M - 57)^+$ ion (m/z 196), very likely a 2-methylthio-4-amino-6-cyclopropylamino-*s*-triazine), resulted from the loss of a *tert*-butyl group with the 4-*tert*-butylamino group in the molecular ion (m/z 253). The m/z 196 ion could further lose a methyl group from the 2-methylthio group to yield the m/z 182 ion, which was likely a 2-thio-4-amino-6-cyclopropylamino-*s*-triazine. The m/z 182 ion could simultaneously lose both a cyclopropyl group with the 6-cyclopropylamino group and a sulphur with the 2-thio group in the triazine moiety to produce the m/z 112 ion, which was a 2,4-diamino-*s*-trizine.

The mass spectrum of the suspected new metabolite M1 formed during the fungal biotransformation of Irgarol 1051 is shown in Figure 4. Without an authentic reference compound, the molecular structure of M1 could only be tentatively deduced based on its molecular ion and the pattern of major fragment ions. It can be seen that M1 had a molecular ion at m/z 213 and with major fragment ions at m/z 198, 157, 110 and 68. The $(M - 15)^+$ ion (m/z 198) was very likely a 2-thio-4-*tert*-butylamino-6-amino-*s*-triazine and resulted from the loss of methyl group with the 2-methylthio group from the molecular ion (m/z 213). The $(M - 56)^+$ ion (m/z 157) was probably a 2-methylthio-4,6-diamino-*s*-triazine and was formed by the loss of a *tert*-butyl group with the 4-*tert*-butylamino group from the molecular ion (m/z 213). A further loss of the 2-methylthio group from the ion m/z 157 produced the ion m/z 110, which was probably a 2,4-diamino-*s*-triazine compound. The relative abundance of the two mass ions m/z 110 and 68 in the mass spectrum (Figure 4) imply that M1 could have a atrazine-like structure, or more correctly a *s*-triazine containing molecular structure. Mougin *et al* (1994) studied biotransformation of the herbicide atrazine using the white rot fungus *P. chrysosporium* and their mass spectral data showed that atrazine, deisopropylatrazine and deethylatrazine all had the prominent m/z 110 and 68 ions. Based on all of the above data, metabolite M1 was hypothesized to be 2-methylthio-4-*tert*-butylamino-6-amino-*s*-triazine.

M1 appeared to be the only major metabolite accumulated during the entire process of Irgarol biotransformation by the white rot fungus *P. chrysosporium*. Figure 5 shows the total ion chromatogram of the DCM extract from a 5-day Irgarol degradation plate. The chromatogram clearly indicates that M1 was a predominant metabolite during the early stage of Irgarol biotransformation. The sequential fungal biotransformation of Irgarol 1051 over an incubation period of 60 days is shown in Figure 6. The results strongly suggested that M1 was a very stable metabolite, perhaps also a terminal metabolite in the fungal biotransformation of Irgarol. The deduction was supported by another experiment in which the incubation of the Irgarol degradation plates was extended to a total period of 6 months and no significant reduction in M1 concentrations in the plates was noted (data not shown).

Based on the limited information obtained in the present study, a proposed metabolic pathway for the new antifouling compound Irgarol 1051 by the white rot fungus *P. chrysosporium* is shown in Figure 7. The pathway includes N-dealkylation of a cyclopropyl group from the cyclopropylamino side chain at the

6-position of the *s*-triazine ring to yield the stable metabolite M1 (2-methylthio-4-*tert*-butylamino-6-amino-*s*-triazine). M1 could not be metabolized further by the *P. chrysosporium* culture and appeared to accumulate as a terminal metabolite. There was no evidence of the heterocyclic ring cleavage of Irgarol. Irgarol 1051 is a *s*-triazine herbicide and therefore it is not surprising that its metabolic pathway follows the general pattern of microbial degradation of chloro-*s*-triazines in which dealkylation was the major and first mechanism involved in the process of biodegradation (Kaufman and Kearney, 1970; Cook, 1987). In general, fungi including *P. chrysosporium* removed the ethyl group of atrazine in preference to the isopropyl group which is branched, thus hindering the microbial degradation (Kaufman and Blake, 1970; Cook, 1987). By the same reasoning, this may also explain why *P. chrysosporium* attacked the 6-cyclopropylamino group of Irgarol which yielded the metabolite M1, and was not capable of attacking the 4-*tert*-butylamino group which has a branched alkyl group with a tertiary carbon. Interestingly, the dealkylated metabolites of atrazine were not extensively degraded further and were found to accumulate in the fungal culture medium, primarily due to the inability of soil fungi and especially *P. chrysosporium* to cleave the *s*-triazine ring of the herbicide atrazine (Wolf and Martin, 1975; Mougin *et al.*, 1994). Since Irgarol and atrazine are both structure-related compounds, it can be speculated that Irgarol 1051 may have an environmental behaviour and impact similar to those of other *s*-triazine herbicides.

In summary, this study has shown that the major route of Irgarol 1051 metabolism in the white rot fungus *P. chrysosporium* is probably via partial N-dealkylation at the cyclopropylamino group which resulted in the 6-amino-*s*-triazine structure with the methylthio group remaining at the 2 position and with the *tert*-butylamino group remaining the 4 position. This led to the formation of a stable and/or terminal metabolite M1 (2-methylthio-4-*tert*-butylamino-6-amino-*s*-triazine). Since metabolite M1 is much more persistant than the parent compound Irgarol 1051, further work is planned to assess its environmental persistance and ecotoxicity.

CONCLUSIONS

Specific conclusions established from the present study are:

1. The white rot fungus *Phanerochaete chrysosporium* was capable of biotransforming the newantifouling compound Irgarol 1051.

2. Metabolism of Irgarol in the fungus appeared to proceed mainly via partial N-dealkylation at the cyclopropylamino group resulting in the formation of metabolite M1.

3. M1 was tentatively identified as 2-methylthio-4-*tert*-butylamino-6-amino-*s*-triazine.

4. M1 appeared to be a stable and/or terminal metabolite. No evidence of the heterocyclic ring cleavage of Irgarol 1051 was observed, thus implying a possibility of its degradation products accumulating in the environment.

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LEGENDS

Fig 1. Chemical structure of Irgarol 1051 and metabolite.

Fig 2. Comparison of NPD chromatograms of extracts from (A) a fungal growth plate i.e., MME plate without Irgarol 1051, and (B) an Irgarol degradation plate i.e., MME plate with Irgarol 1051. The asterisk indicates normal growth metabolites derived from the metabolism of organic nutrients in the fungal growth medium.

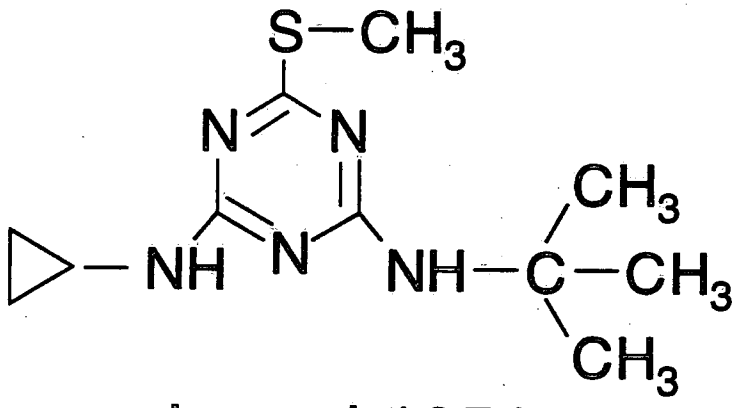
Fig 3. Low-resolution mass spectrum of Irgarol 1051.

Fig 4. Low-resolution mass spectrum of the metabolite M1 from peak M1 in Figure 2B.

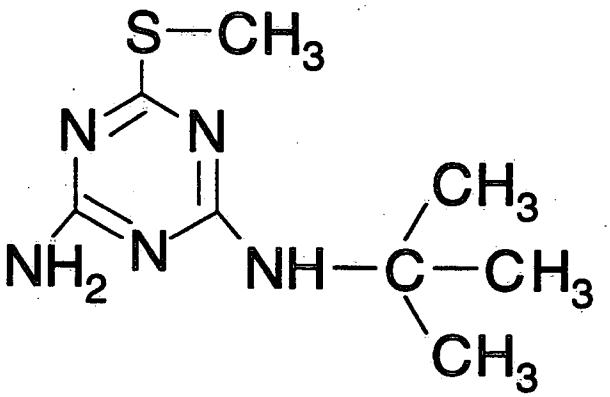
Fig 5. Total ion chromatogram of the DCM extract from a 5-days Irgarol degradation plate.

Fig 6. DCM extracts of fungal growth on Irgarol degradation plates.

Fig 7. Proposed metabolic pathway of Irgarol 1051 by the white rot fungus *Phanerochaete chrysosporium.*

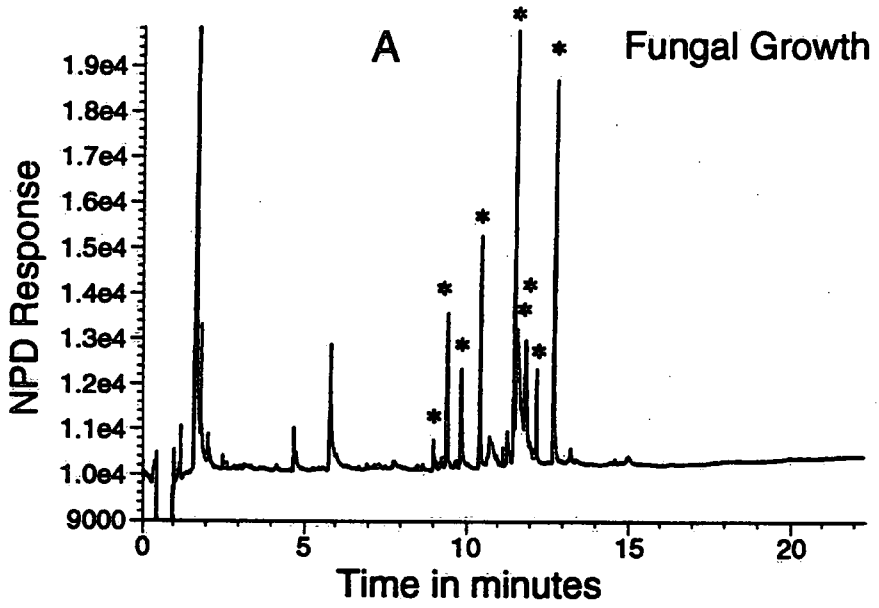


Irgarol 1051



metabolite MI

Figure 1


A
Fungal Growth
NPD Response
1.9e4
1.8e4
1.7e4
1.6e4
1.5e4
1.4e4
1.3e4
1.2e4
1.1e4
1.0e4
9000
0
5
10
15
20
Time in minutes

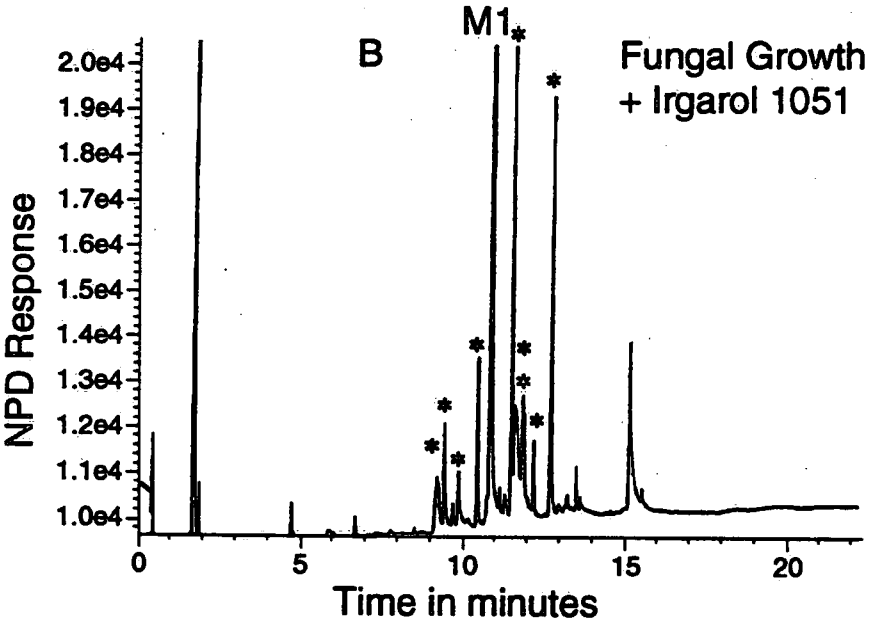

M1
B
Fungal Growth
+ Irgarol 1051
NPD Response
2.0e4
1.9e4
1.8e4
1.7e4
1.6e4
1.5e4
1.4e4
1.3e4
1.2e4
1.1e4
1.0e4
0
5
10
15
20
Time in minutes

Figure 2

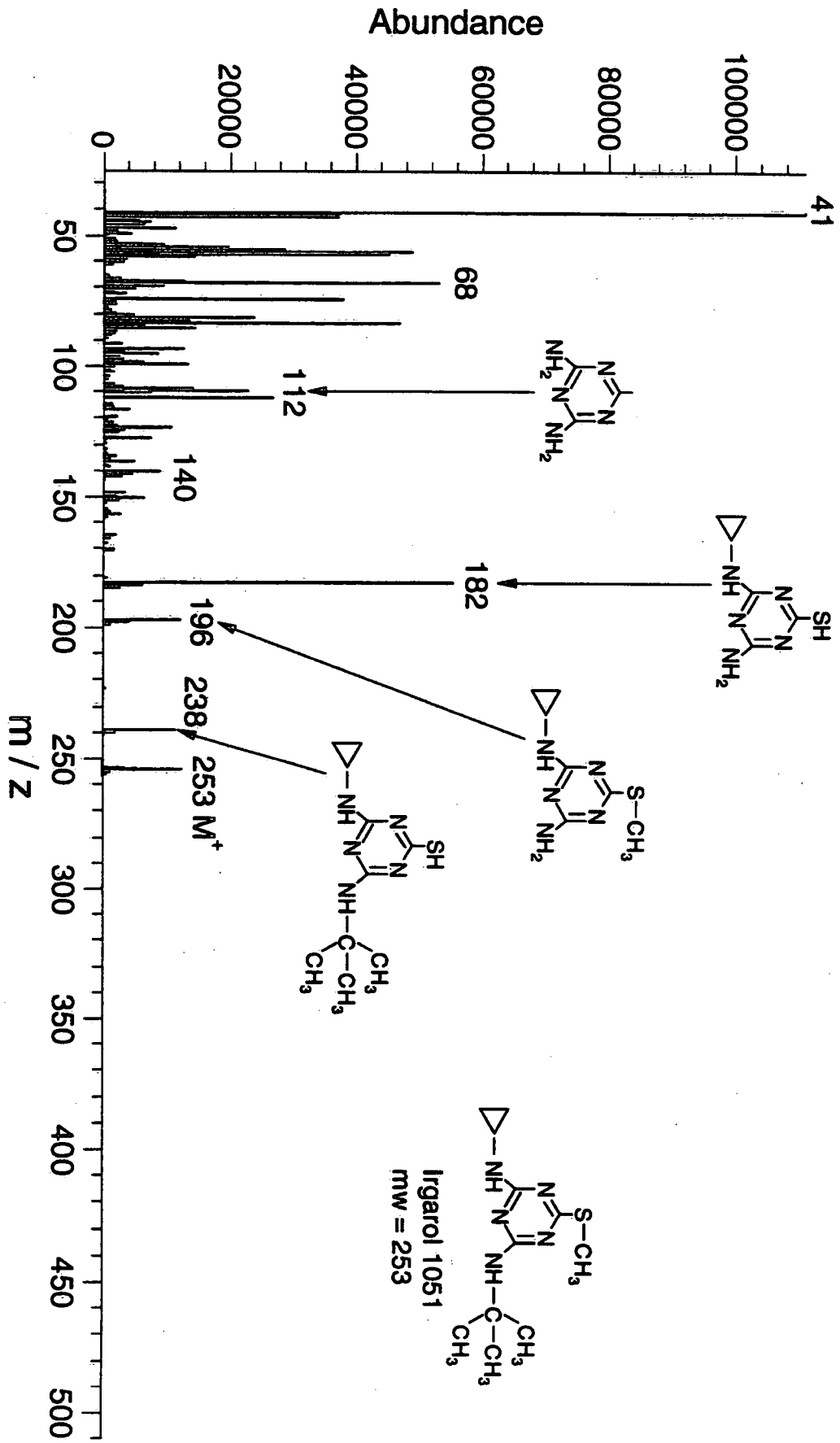

Abundance
0
20000
40000
60000
80000
100000
m / z
50
100
150
200
250
300
350
400
450
500
41
68
112
140
182
196
238
253 M+
Irgarol 1051
mw = 253

Figure 3

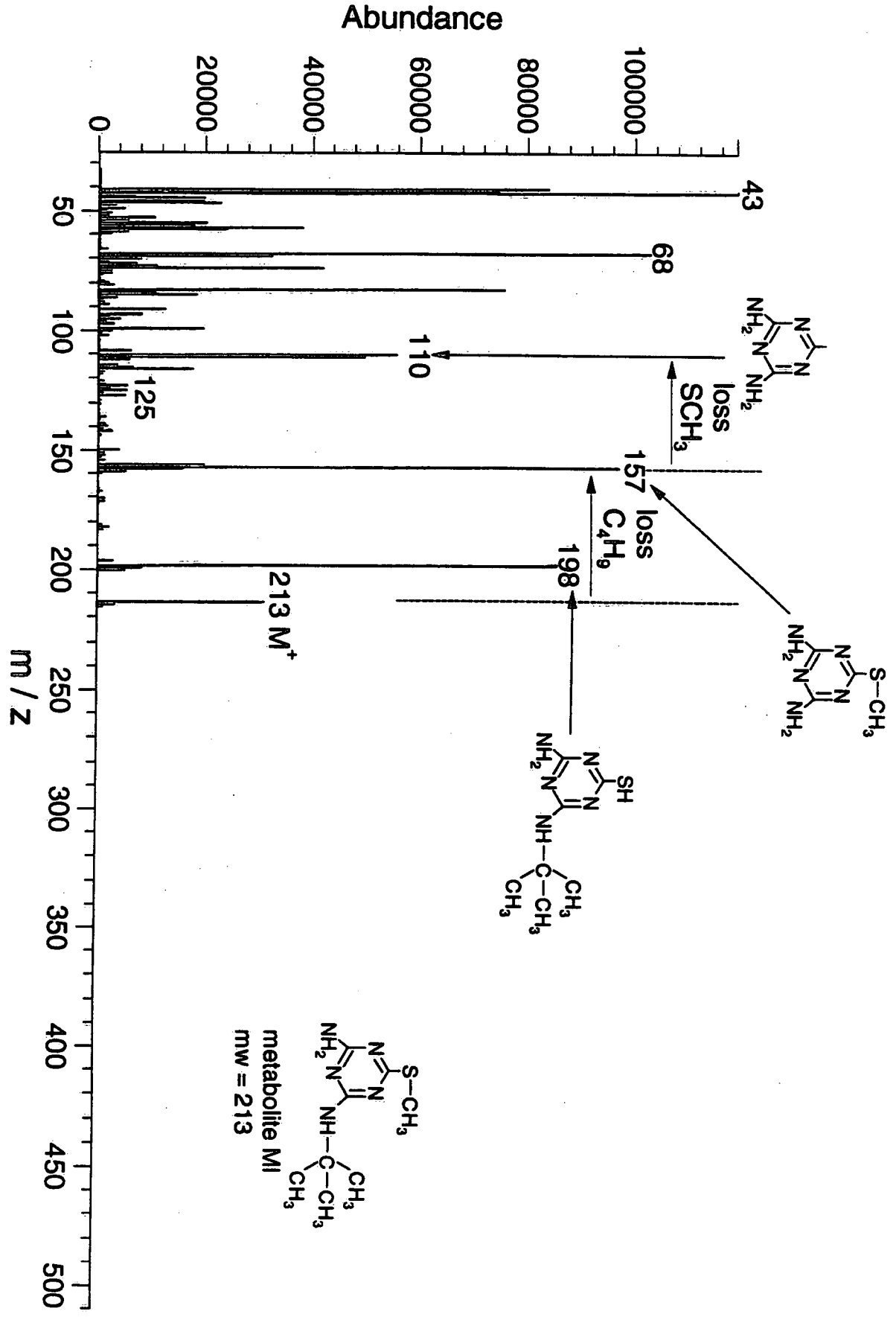

Abundance
0
20000
40000
60000
80000
100000
m / z
50
100
150
200
250
300
350
400
450
500
43
68
110
125
157
198
213 M+
loss SCH3
loss C4H9
metabolite MI
mw = 213

Figure 4

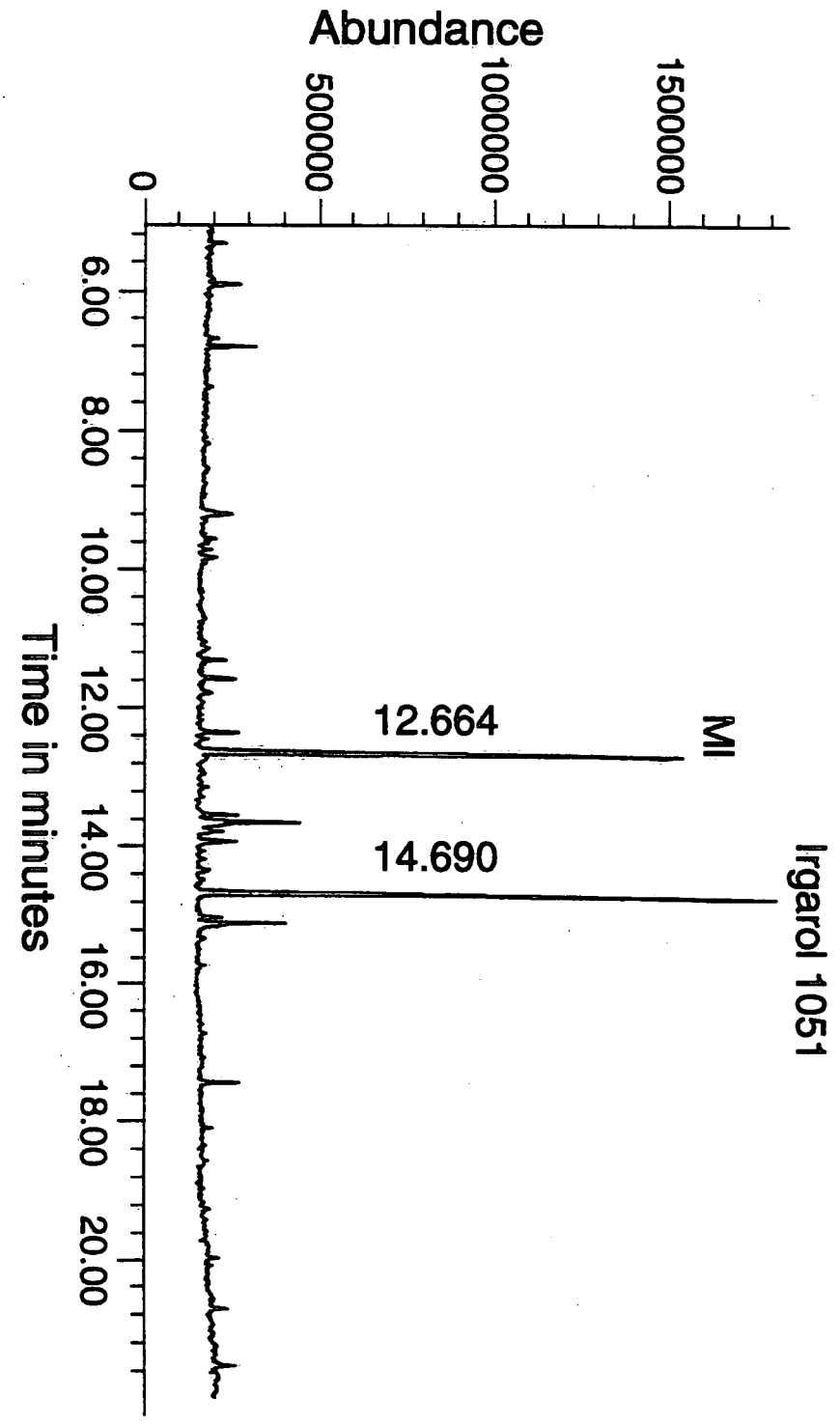

Abundance
500000
1000000
1500000
0
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
Time in minutes
12.664
MI
14.690
Irgarol 1051

Figure 5

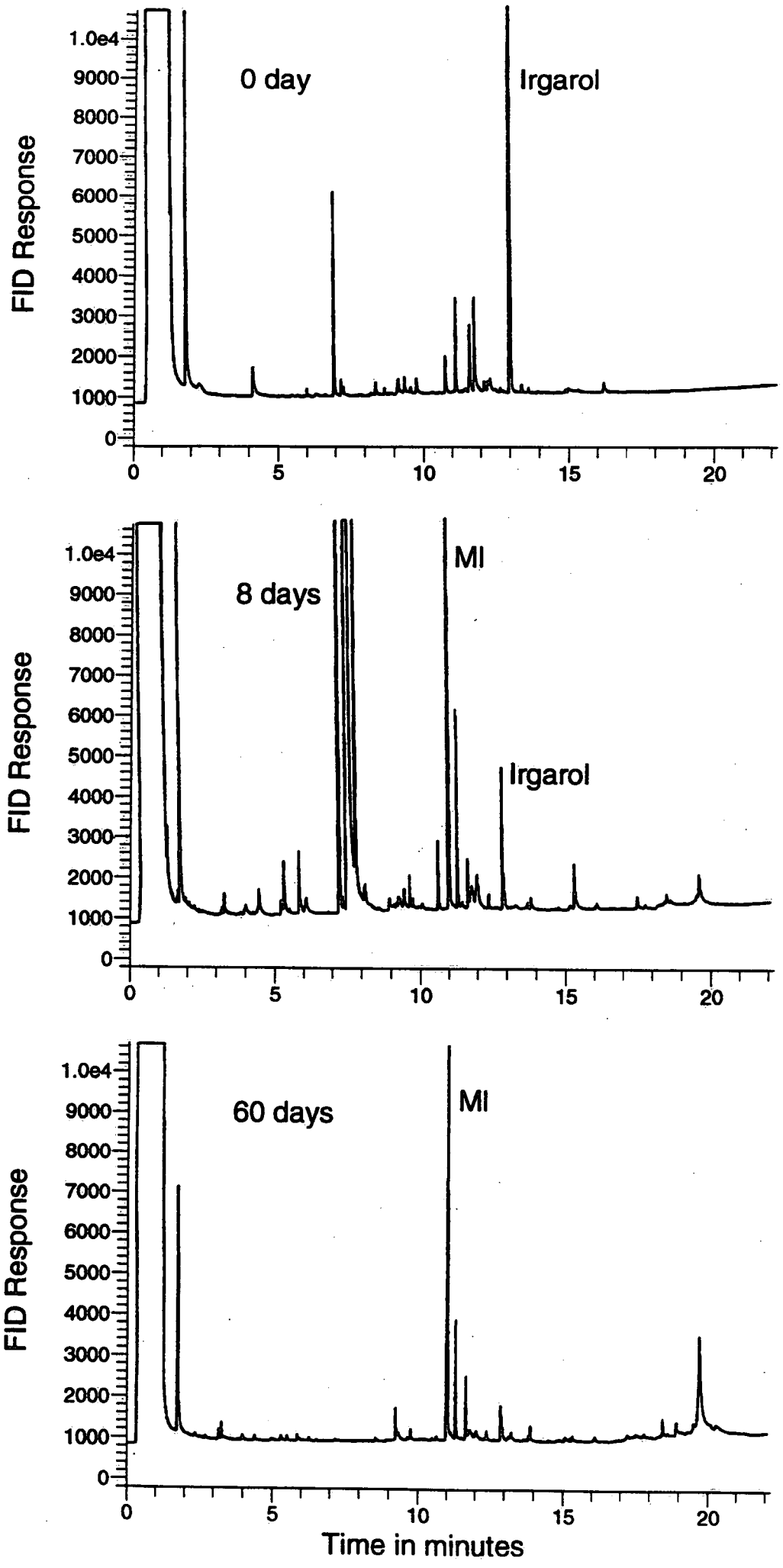
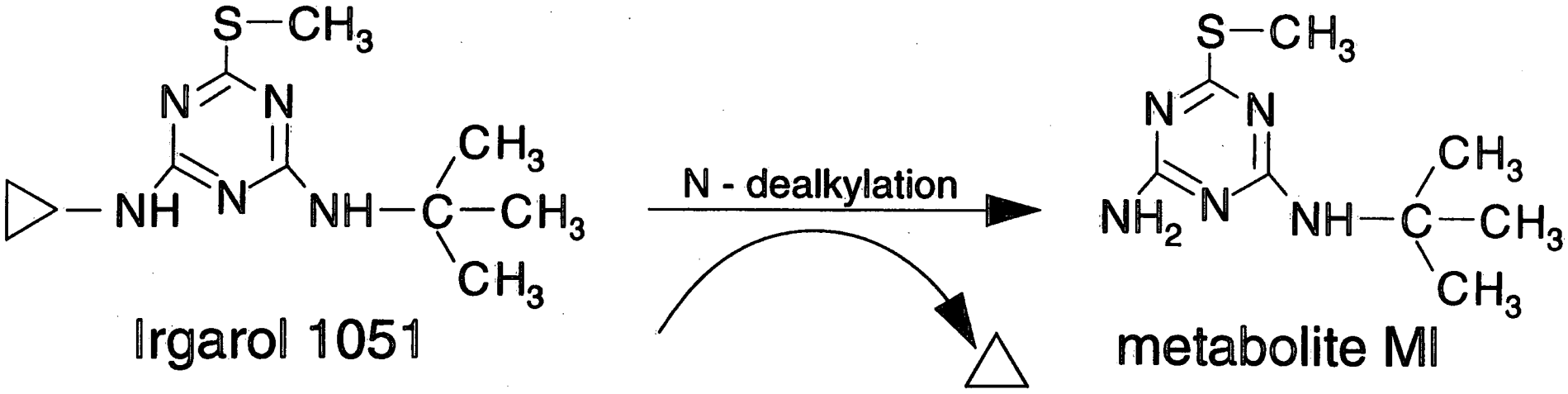

0 day
Irgarol
8 days
MI
Irgarol
60 days
MI
FID Response
FID Response
FID Response
1.0e4
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0
5
10
15
20
Time in minutes

Figure 6

Figure 7


S—CH3
N
N
CH3
NH
N
NH—C—CH3
CH3
Irgarol 1051
N - dealkylation
S—CH3
N
N
CH3
NH2
N
NH—C—CH3
CH3
metabolite MI

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Environment Canada Environnement Canada

Canada