ENVIRONMENT CANADA
CONSERVATION AND PROTECTION
ENVIRONMENTAL PROTECTION
PACIFIC AND YUKON REGION

LEACHING OF 2-(THIOCYANOMETHYLTHIO)BENZOTHIAZOLE (TCMTB) FROM SURFACE TREATED LUMBER

REGIONAL PROGRAM REPORT 90-06

BY

PETER K. KRAHN

MAY 1990

EXECUTIVE SUMMARY

The chemical, 2-(thiocyanomethylthio)benzothiazole (TCMTB) is a fungicide registered for use as an antisapstain chemical to protect dimensioned lumber during transport to export markets. TCMTB is highly toxic to fish and has a 96 hour flow through fish bioassay LC50 of approximately 15 ug/L (parts per billion or ppb) for chinook salmon in freshwater and 6 ppb in sea water.(6) The leaching of this chemical from treated wood was monitored during rainfall events at a sawmill located on the tidally influenced section of the North Arm of the Fraser River from October 1989 to January 1990.

The objectives of this study were:

1) to determine the concentrations of TCMTB and its degradation products in rainwater leachate from surface treated lumber;

2) to determine the concentrations of TCMTB and its degradation products in the Fraser River adjacent to storm water outfalls, which drain TCMTB treated lumber storage yards.

Various samples were collected including rainwater, leachate dripping directly from treated lumber and runoff discharging from the treated lumber storage yard. Fraser River water was sampled at various locations and depths adjacent to the discharge during periods of rainfall. The samples were collected as half hour or one hour composites and analysed by High Peformance Liquid Chromatography (HPLC) for the presence of TCMTB, 2-(Methylmercapto) benzothiazole (MMBT), 2-Mercaptobenzothiazole (MBT), and Benzothiazole (BT).

The sawmill used state-of-the-art high pressure longitudinal and cross chain spray box systems which had been modified to apply an average 61 +/- 20 ug TCMTB/cm^2 or 30% more than the recommended rate of 40 ug/cm^2. Treatment was applied to rough and planed lumber. The modification was made prior to the initiation of the study in response to a claim against lumber, which had been shipped overseas. The concentration of the working solution in one of the

three mixrooms averaged 4,344,000 ppb TCMTB and was found to vary from 2,230,000 ppb to 10,100,000 ppb. This fluctuation was traced to the random addition of wash water as makeup water in the mixtank. Flow metering equipment was installed to correct this problem part way through the study. The observations made during this study indicate that;

1) A comparison between the dosage of TCMTB at this mill which uses high pressure spray technology and another mill which uses dip treatment technology indicates that when TCMTB is allowed more than 12 hours drying time the leach rate is directly proportional to the retention. The concentration of TCMTB in the runoff from a treated lumber storage yard averaged over an entire storm event may be estimated by the equation:

$$y = \frac{95.5(x)}{(15 \pm 9)}$$

"y" is the concentration of TCMTB in the runoff in ug/L and,
"x" is the loading rate of TCMTB in ug/cm^2.

95.5 is the estimated leaching rate vs chemical retention factor for TCMTB.
15 ± 9 is the dilution range which occurs at most sawmills.

Mills which achieve the recommended retention of 40 ug/cm^2 of TCMTB are estimated to have an average yard runoff concentration of 255 ug/L. Factors specific to an individual mill will cause the TCMTB concentrations in yard runoff to range from 160 to 640 ug/L. This equation indicates that a lumber treatment facility designed to comply with the current code of practice for TCMTB will not be able to comply with the British Columbia Antisapstain Chemical Waste Control regulation limit of 15 ppb or satisfy the intent of the Canada Fisheries Act. Further improvements are required. After achieving a minimum of 2 1/2 hours of covered storage the most viable improvement is likely to be a reformulation of the TCMTB antisapstain product to increase the retention of the chemical in the wood.

2) High pressure TCMTB treated planed lumber, with less than one hour of covered storage, leached at an average concentration of 89,000 ppb and as a result of dilution by rain discharged yard runoff to the storm sewer at an average concentration of 7,500 ppb.

3) High pressure TCMTB treated planed lumber, with at least 12 hours of covered storage, leached at an average concentration of 5433 +/- 1944 ppb TCMTB and discharged to sewer at an average concentration of 340 ppb. This is a 94% reduction in average concentration compared to lumber which had less than one hour of covered storage after treatment.

4) High pressure TCMTB treated lumber requires more than 11 minutes of covered storage immediately after treatment to prevent excessive leaching of chemical.

This covered storage time is required for all treatment methods including dip, low and high pressure spray. The current formulations will have to be modified so that leachability of the active ingredients are reduced to comply with the requirements of the Fisheries Act and the B.C. regulation.

5) The concentration of TCMTB in the stormwater runoff from the treated lumber storage yard was inversely proportional to the intensity of the rainfall. The runoff concentration at this mill ranged from 185 to 533 ppb TCMTB. The concentration of TCMTB in the runoff increases once the rainfall has ended due to a reduced dilution of leachate dripping from the treated lumber.

6) TCMTB on lumber that has had prolonged exposure to sunlight and rainfall may degrade to 2-mercaptobenzothiazole (MBT). The 96 hour LC50 of MBT compound is 730 ppb for rainbow trout fingerlings.(4) In one sample of leachate (from lumber which had a longer than normal storage period on the mill yard) MBT was analysed at 4,740 ppb in leachate dripping from the lumber and at 1220 ppb in effluent discharging from the storage yard into the Fraser River. Information on the toxicity to Chinook salmon was not available. Further study of MBT concentrations are required to determine if MBT should be included in effluent regulations.

7) A sawmill located on the lower Fraser River, which has from one to four hours covered storage immediately after treatment and has an uncovered yard inventory of 6,000,000 board feet of lumber treated with TCMTB will discharge an average of 450,000 L/d of contaminated stormwater effluent on at least 30 days per year and 700,000 L/d of effluent on at least 5 days per year. The average effluent concentration will depend on the chemical application rate and under normal conditions is estimated to range from 240 ppb to 340 ppb. This is 16 to 23 times the allowable concentration in the B.C. Provincial Antisapstain Chemical Waste Control Regulation.

8) The quantity of TCMTB discharged from this mill into the Fraser River will range from 90 to 250 grams per rainfall event.

9) The effluent plume discharged from a sawmill in the tidally influenced zone on the lower Fraser River stratified in the top meter of the river regardless of the tidal cycle. The plume will create a high toxicity zone extending 10 meters from the discharge on at least 30 days per year and 10 to 20 meters at least 5 days per year. Within these distances the concentration of TCMTB will be three to five times the regulated limit of 15 ppb for a period of five to ten hours. (Or as long as rainfall continues.) This area is a particularly sensitive environment as juvenile salmonids frequent the surface and the shore during the rearing stage of their lifecycle.

RESUME

Le produit chimique, 2-(thiocyanométhylthio)benzothiazole (TCMTB), est un fongicide homologué utilisé comme produit anti-tache pour protéger le bois débité au cours du transport vers les marchés d'exportation. Le TCMTB est trés toxique pour le poisson et a une CL50 de bio-essai à renouvellement continu à 96 heures d'environ 15 ug/L (parties par milliard) pour le saumon quinnat en eau douce, et de 6 parties par milliard dans 'leau de mer.(6) Le lessivage de ce produit chimique provenant du bois traité a fait l'objet d'une surveillance lors des précipitations dans une scierie située dans une section du North Arm de fleuve Fraser influencée par la marée, d'octobre 1989 à janvier 1990.

Cette étude avait comme objectifs:

1) Le dosage des concentrations de TCMTB et de ses produits de dégradation dans le lixiviat des eaux de pluie provenant du bois traité en surface.

2) Le dosage des concentrations de TCMTB et de ses produits de dégradation dans les eaux du fleuve Fraser dans le voisinage des exutoires des égouts pluviaux qui drainent les terrains d'entreposage du bois traité au TCMTB.

Divers échantillons ont été prélevés, notamment de l'eau de pluie, des lixiviats dégouttant directement du bois traité, et des eaux de ruissellement évacuées du terrain d'entreposage du bois traité. Les eaux de fleuve Fraser ont été échantillonnées à diverses profondeurs, à plusieurs endroits adjacents aux points d'évacuation durant les périodes de précipitation. On a prélevé des échantillons composites d'une demi-heure et d'une heure qui ont été analysés à l'aide de la chromatographie liquide à haute performance (CLHP) pour déceler la présence de TCMTB, de 2-(Méthylmercapto)benzothiazole (MMBT), de 2-Mercaptobenzothiazole (MT) et de Benzothiazole (BT).

La scierie utilisait des systèmes perfectionnés de boites de pulvérisation haute pression, à chaine longitudinale et transversale, qui

avaient été modifiés pour appliquer en moyenne 61 $\pm$ 20 ug de TCMTB/cm^2, soit 30% de plus que le taux d'application recommandé de 40 ug/cm^2. Le traitement était appliqué à du bois non raboté et du bois raboté. Le procédé a été modifié avant le début de l'tude, suite à une plainte relative à du bois qui avait été expédié outre-mer. La concentration de la solution de travail dans une des trois salles de mélange était en moyenne de 4 344 000 parties par milliard de TCMTB, variant de 2 230 000 à 10 100 000 parties par milliard. On a découvert que cette fluctuation provenait de l'ajout au hasard d'eau de lavage comme eau d'appoint dans le réservoir de malaxage. Un débimètre a donc été installé, une fois l'étude commencée, pour corriger ce problème. Les observations faites au cours de l'étude sont les suivantes:

1) Une comparaison entre le dosage de TCMTB à cette scierie qui utilise une technique de vaporisation haute pression, et une autre scierie qui utilise une technique de traitement par trempage indique que, lorsqu'on laisse le bois traité au TCMTB sécher pendant plus de 12 heures, la vitesse de lessivage est directement proportionnelle au temps de rétention. La moyenne de la concentration de TCMTB dans les eaux de ruissellement de terrain d'entreposage du bois traité durant toute une tempête peut être calculée à l'aide de l'èquation suivante:

$$y = \frac{95.5(x)}{(15 \pm 9)}$$

"y" est la concentration de TCMTB dans les eaux de ruissellement, en ug/L, et
"x" est le taux de charge de TCMTB en ug/cm^2.

95.5 est la vitesse estimative de lessivage par rapport au facteur de rétention de produit chimique pour le TCMTB.

15 ± 9 est la plage de dilution dans la plupart des scieries.

La concentration moyenne des eaux de ruissellement de terrain d'entreposage des scieries qui obtiennent la rétention recommandée de 40 ug/cm^2 de TCMTB est censée être de 255 ug/L. Les facteurs particuliers à une scierie donnée font varier les concentrations de TCMTB dans les eaux de ruissellement du terrain d'entreposage de 160 à 640 ug/L. Cette équation indique qu'une installation de traitement de bois, conçue de façon à se conformer au code de pratique actuel en matière de TCMTB, ne pourra pas se conformer à la limite de 15 parties par milliard du Antisapstain Chemical Waste Control Regulation de la Colombie-Britannique ni respecter l'intention de la loi fédérale sur les pêches. D'autres améliorations sont nécessaires. Après avoir obtenu une durée minimale de 2 1/2 heures en entreposage dans un endroit couvert, l'amélioration la plus rentable est probablement la reformulation de produit anti-tache à base de TCMTB de façon à ce qu'il adhère mieux au bois.

2) La concentration moyenne des eaux de lessivage du bois raboté, traité à haute pression avec du TCMTB, entreposé une heure dans un endroit couvert, était de 89 000 parties par milliard, donnant ainsi une concentration moyenne de 7 500 parties par milliard après dilution par les pluies, dans les eaux de ruissellement évacuées dans les égouts pluviaux.

3) Après lessivage, la concentration moyenne de TCMTB dans les eaux de ruissellement du bois raboté traité à haute pression, entreposé pendant au moins 12 heures dans un lieu couvert, était de 5 433 $\pm$ 1 944 parties par milliard, et la concentration moyenne de ce produit dans les eaux rejetées dans les égouts était de 340 parties par milliard. Il s'agit d'une réduction de 94% de la concentration moyenne comparativement au bois entreposé pendant moins d'une heure dans un lieu couvert après traitement.

4) Le bois traité à haute pression au TCMTB doit être entreposé pendant plus de 11 minutes dans un endroit couvert, immédiatement après le traitement, afin d'éviter un lessivage excessif du produit chimique.

Cette durée de l'entreposage à couvert est nécessaire pour toutes les méthodes de traitement, y compris le trempage et la pulvérisation à basse et haute pression. Les formules actuelles devront être modifiées afin de réduire les caractéristiques de lessivage des ingrédients actifs de façon à se conformer aux exigences de la Loi sur les pêches et du règlement de la Colombie-Britannique.

5) La concentration de TCMTB des eaux de ruissellement pluviales provenant de terrain d'entreposage du bois traité était inversement proportionnelle à l'intensité des averses. La concentration des eaux de ruissellement de cette scierie variait de 185 à 533 parties par milliard de TCMTB. La concentration de TCMTB dans les eaux de ruissellement augmente lorsque la pluie a cessé en raison d'une dilution réduite des lixiviates qui s'égouttent du bois traité.

6) Le TCMTB qui a servi au traitement de bois exposé pendant de longues périodes au soleil et à la pluie peut se dégrader en 2-mercaptobenzothiazole (MBT). La CL50 après 96 heures d'exposition au MBT est de 730 parties par milliard chez les jeunes truites arc-en-ciel.(4) Dans un échantillon de lixiviat provenant de bois qui avait été entreposé plus longtemps que la normale sur le terrain d'entreposage, on a trouvé 4 740 parties par milliard de MBT dans les lixiviats qui s'égouttaient du bois, et 1 220 parties par milliard dans les effluents évacués dans les eaux de Fraser. Il n'y avait pas d'information sur la toxicité de ce produit pour le saumon quinnat. D'autres études sur les concentrations de MBT sont nécessaires pour savoir si le MBT doit être inclus dans les règlements sur les effluents.

7) Une scierie située dans le cours inférieur de fleuve Fraser, où le bois est entreposé dans un endroit couvert pendant une période d'une à quatre heures immédiatement après le traitement, et qui possède un inventaire de 6 millions de pieds linéaires de bois traité au TCMTB, déversera chaque jour en moyenne 450 000 litres d'eaux pluviales contaminées, pendant au moins 30 jours par année, et 700 000 litres par jour d'effluents, au moins 5 jours par année. La concentration moyenne des effluents dépendra du taux d'application de produits chimiques, et dans des conditions normales, cette concentration devrait varier de 240 à 340 parties par milliard, soit 16 à 23 fois la concentration permise par le Antisapstain Chemical Waste Control Regulation de la Colombie-Britannique.

8) La quantité de TCMTB évacuée à partir du site de cette scierie dans le fleuve Fraser variera de 90 à 250 grammes par précipitation.

9) Le panache des effluents évacués par une scierie dans une zone du fleuve Fraser influencée par les marées se stratifie dans le premier mètre des eaux de fleuve, quel que soit le cycle de marée. Le panache créera une zone très toxique qui s'étendra à 10 mètres du point d'évacuation, au moins 30 jours par année, et de 10 à 20 mètres du point d'évacuation, au moins 5 jours par année. Dans ces zones, la concentration de TCMTB sera 3 à 5 fois supérieure à la limite permise de 15 parties par milliard pendant une période de 5 à 10 heures. (Ou aussi longtemps que durera l'averse.) Cette région est un milieu particulièrement sensible étant donné que les salmonidés juvéniles fréquentent les eaux de surface et le littoral au cours de l'étape de l'alevinage de leur cycle biologique.

TABLE OF CONTENTS

PAGE

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

1.0 OBJECTIVES

The objectives of this study were:

1) To determine the concentrations of 2-(thiocyanomethylthio)-benzothiazole (TCMTB) and related benzothiazoles in rainwater leachate from surface treated lumber.
2) To determine the concentrations of TCMTB and its metabolites in the Fraser River adjacent to storm water outfalls which drain TCMTB treated lumber storage yards.

To achieve these objectives a representative sawmill using state-of-the-art equipment to apply TCMTB as an antisapstain chemical was selected for detailed study. The mill was located on the tidally influenced portion of the lower Fraser River. The study period was from October 1989 to January 1990 and encompassed nine major rainfall events.

2.0 BACKGROUND INFORMATION

In Canada, TCMTB is registered as an active antimicrobial ingredient in 19 products. Eleven of the registrations are for antisapstain formulations.(4)

2.1 Synthesis of TCMTB and Its Chemical Properties

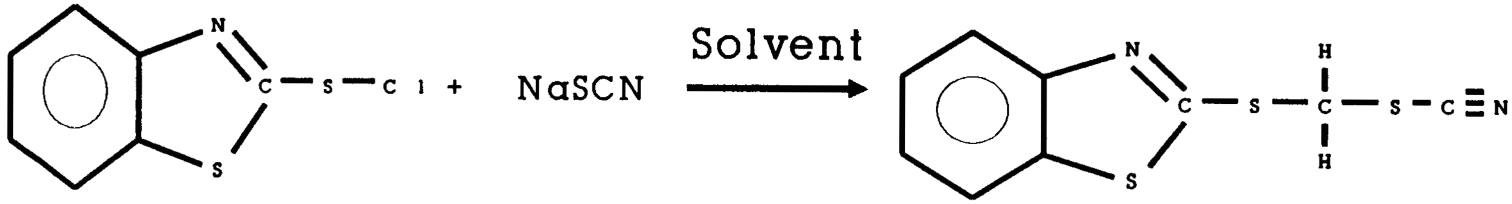
A mixture of chloromethylthiobenzothiazole (CMTC) with sodium thiocyanate in the presence of a solvent will produce 2-(thiocyanomethylthio) benzothiazole or TCMTB(5). (See Figure 1). 2-mercaptobenzothiazole (MBT) is also a major reactant in the formulation of TCMTB(4). Reaction byproducts include residual CMTC and MBT and hydrogen cyanide gas(1,4). The major reaction and decomposition compounds associated with TCMTB are listed in Figure 2.

In humans, TCMTB is a strong skin, eye and mucus membrane irritant. At doses of greater than 33 mg/kg body weight/day it can be a stomach irritant. Chronic toxicity and carcinogenicity tests are currently underway and mutagenicity studies in rats, hamsters, and mice showed no negative effects(4).

Teratology (birth defects) data was limited and only reported for rabbits dosed at 20-40 mg/kg body weight. A rat metabolism study indicated that 80% of the ingested material was excreted in 24 hours in the urine(4).

The hydrolysis of TCMTB is pH dependent, i.e. at pH 5 no significant effect was observed and at pH 9 hydrolysis was quite rapid with a half life (t1/2) of several days(1,4). (See Section 6).

Photolysis of TCMTB yields MBT and is a likely route of dissipation in surface waters(4). Products of photolysis include MBT and benzothiazole (BT). (See Section 6).



chloromethylthio-
benzothiazole (CMTC)

2-(thiocyanomethylthio)
benzothiazole (TCMTB)

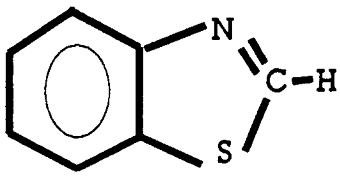
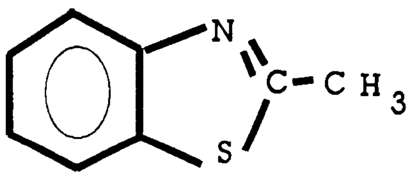
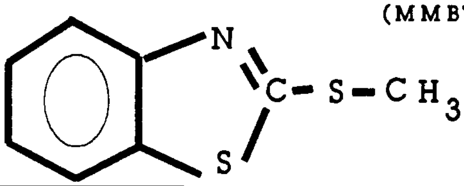
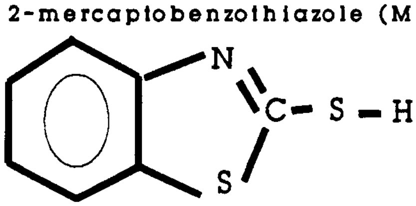
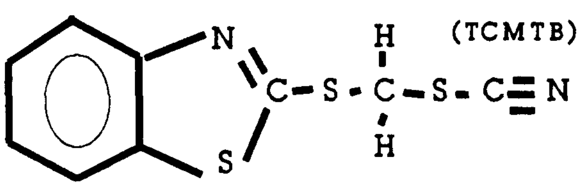
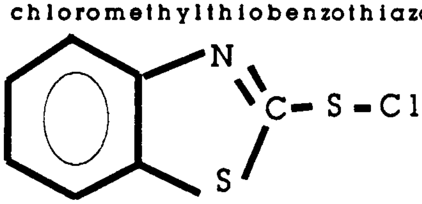
+ NaCl

+ CMTC (residual, see (4))

+ $HCN_{(gas)}$
is evolved in storage
containers and in use

Figure #1: Reaction Mechanism for the Production of TCMTB

Figure #2: Benzothiazole Compounds Related to TCMTB Production or Decomposition

Compound	Water Solubility at 24 C	Phase at 24 C
benzothiazole (BT) 	3000 mg/L	Liquid/Polar
2-methylbenzothiazole (MeBT) 	2300 mg/L	Liquid/Polar
2-(methylmercapto)benzothiazole (MMBT) 	125 mg/L	Solid
2-mercaptobenzothiazole (MBT) 	120 mg/L 54 mg/L @ 5C	Solid
2-(thiocyanomethylthio)benzothiazole (TCMTB) 	40 mg/L 16 mg/L @ 5C	Solid
chloromethylthiobenzothiazole (CMTC) 	?	?

Biotransformation of TCMTB is believed to occur with an estimated half life (t1/2) of 5 days in soil. MMBT is believed to be a product of biological methylation and along with BT may be bioaccumulative and has been found in extracts of liver of starry flounder(2).

A wood mixture containing 500 ppm of TCMTB has been shown to undergo complete destruction by combustion in hog fuel boilers with no potential for generation of dioxins or furans. Sulfur dioxide was a combustion product(4).

The biological toxicity of TCMTB is dependent on the species, pH and salinity of the water. TCMTB was found to be non-toxic to mallard ducklings with the estimated LD50 >10,000 ppm. It is highly toxic to juvenile fish with static 96h LC50 values of 39-54 ppb for rainbow trout and 15-18 ppb for chinook salmon.(4) The major transformation product of TCMTB is MBT which has a static 96h LC50 of 730 ppb to rainbow trout fingerlings(4). Flow through bioassays using this compound on rainbow trout fingerlings indicated LC50 values of 1140, 730 and 670 ppb for 24, 96, and 192 hours(4).

The mobility of TCMTB in soil is dependent on the organic content. Low mobilities were observed in soils with greater than 2% organics and high mobilities were found in soils such as sand which had less than 0.8% organics(4). TCMTB has been shown to penetrate asphalt in high contact operations such as the dripping pads at diptanks(7).

The solubility of TCMTB in water is temperature dependent and was found to be 16 mg/L at 5 Celcius and 40 mg/L at 24 Celcius. The chemical structures and solubilities of the various related benzothiazole compounds and their chemical structures are listed in Figure 2.

2.2 Description of the Sawmill Lumber Production Process

The mill is situated on a 7 ha (17.3 acres) site with a sawmill and a segregated rough cut and finished lumber storage yard. The drainage from each yard was segregated and discharged directly to the foreshore of the Fraser

River. A city storm sewer passed through the yard but does not accept runoff from the yard (Figure 3).

Lumber at the study mill was predominantly spruce/pine/fir (SPF) which was treated in high pressure spray booths that have a chemical delivery line pressure of 827,000 Pa (8.2 atmospheres). Each spray booth consisted of a lower bank of 19 nozzles and an upper bank of 15 nozzles with 0.79 mm (0.031 inch) diameter orifices. This mill had three spray systems serving a rough cut heavy timber line, a rough cut dimensioned lumber line and a planed dimensioned lumber line.

The heavy timber line produced lumber with a rough surface which was sprayed in a longitudinal spray box. The lumber exited the spray box via a conveyor to an automated sorting system which was exposed to precipitation. Covered storage on this line was less than one minute. The lumber was removed from this sorting system via forklift and stored in a covered sorting area. This design was unique to the mill and thus no sampling was conducted here.

Lumber was cut to a rough dimension and sprayed in a high pressure cross chain spray booth. Upon exiting the spray booth the rough cut lumber was automatically sorted into covered storage bins. Residence time in the covered bins varied up to 10 hours. Rough cut lumber was removed from the storage bins, automatically stacked and stored on asphalt in an uncovered yard.

Rough cut lumber is removed from the storage yard after an average of 30 days (some dimensional lumber may be 3-4 months) and processed through a cross chain planer followed by a cross chain high pressure spray system. The boards are planed on two sides with the other two sides remaining as rough surfaces. The unplaned sides will therefore receive a second treatment with TCMTB while the planed surfaces receive a single treatment. The lumber exiting from the spray system is split into two lines. One line goes to a covered packaging area to be strapped and spray painted with end sealer. End

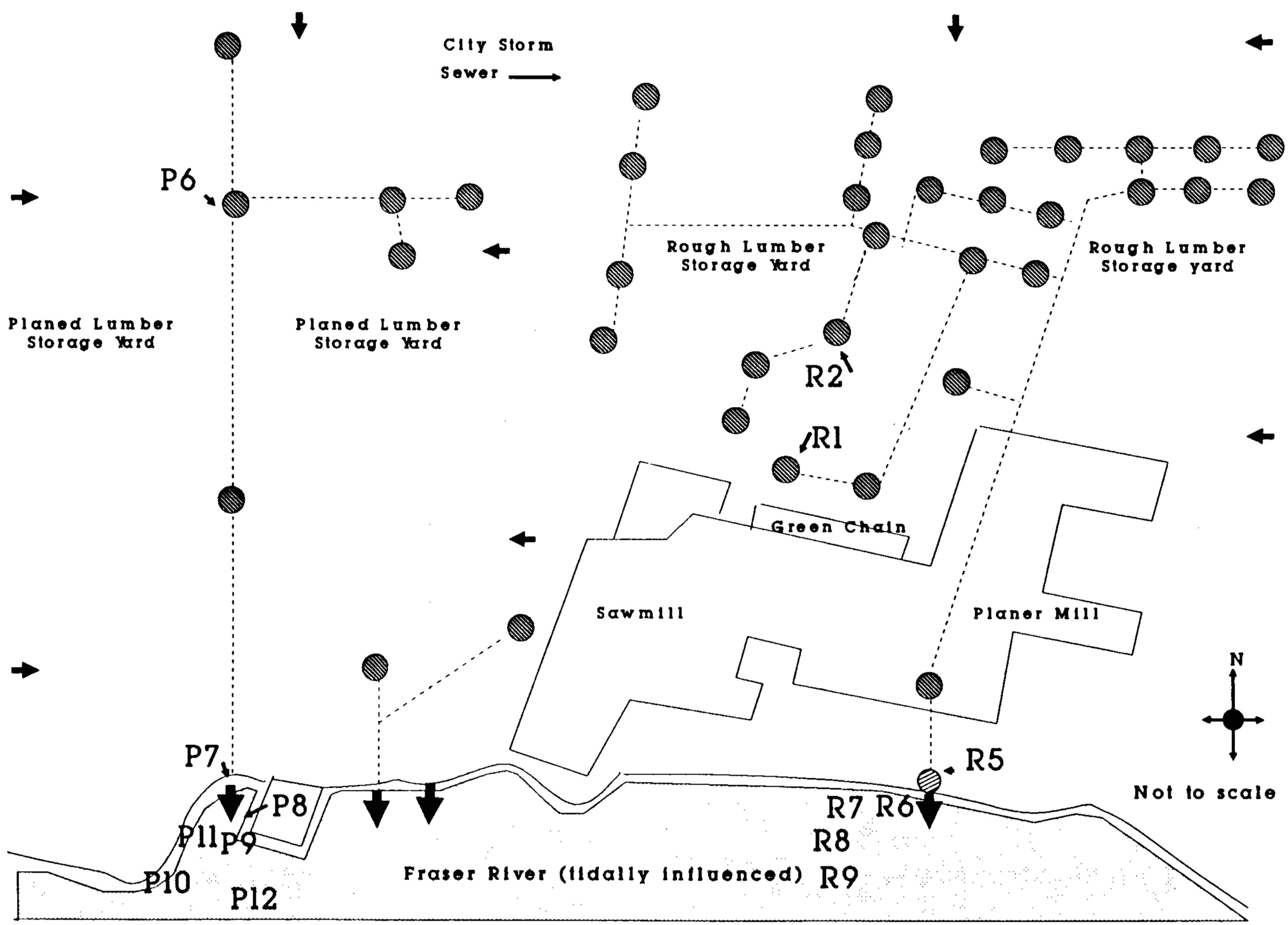


Figure #3: Sawmill Site Map with Storm Drainage System sealing is used to prevent checking (cracking of lumber at the ends due to rapid drying). Lumber on this line remains under cover up to several hours after treatment. The second line of lumber passes to a covered sorting conveyor where it has a residence time of one to seven minutes under covered storage prior to exposure to precipitation. This lumber is stacked by hand according to size and grade and is considered freshly treated lumber.

3.0 FIELD METHODS

All samples were collected in heat treated pesticide grade amber glass jars with heat treated foil liners and plastic lids. Five types of samples were collected consisting of rainwater, working solution, leachate, runoff and Fraser River water.

3.1 Collection of Rainwater

Rainfall at the mill site was monitored using a "TRU-CHEK" rain gauge. Total accumulated rainfall was recorded at each sampling sequence. (Plate #1) The spray booth which applies TCMTB to the lumber was exhausted via a demister, through the roof directly above it. Samples of rainwater were collected from down spouts which drain the roof. (TCMTB was not detected in rainwater).

3.2 Sampling of the Working Solution

Working solution was collected directly from the mix tank in an amber glass jar as a single grab sample. Samples were collected at random during the sampling sequence.

3.3 Sampling of the Leachate from Freshly Treated Lumber

In this study, leachate was defined as rainwater which dripped directly from treated lumber before striking the paved surface of the storage area.

The uneven lengths of boards in the freshly treated lumber stacks created spaces between them into which rectangular 3.0 L clear glass heat treated pyrex baking trays are placed.(Plate #2,#14) The trays remained in the stack until the sampling sequence was complete or the lumber stacks were moved to the storage yard.

PLATE #1 TRUCHECK rainguage used to monitor rainfall

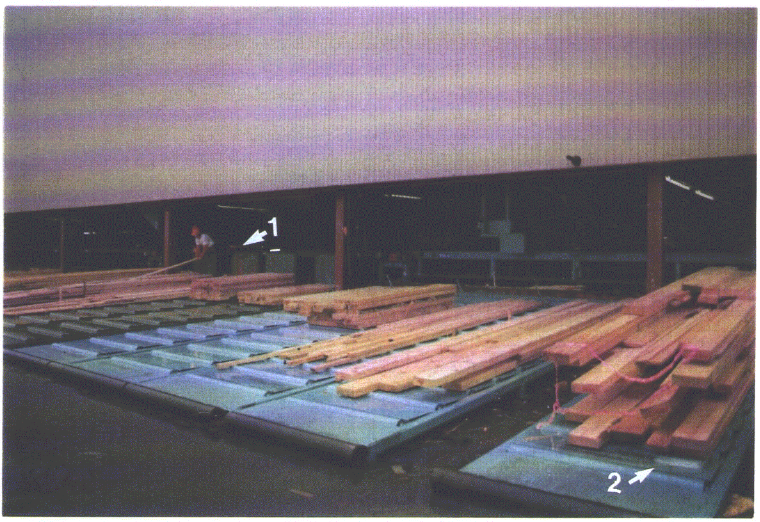


PLATE #2 Planed lumber being removed from the green chain (#1) after seven to eleven minutes of covered storage. (#2) Heat treated pyrex tray which collects leachate from treated lumber

3.4 Sampling of Leachate from Long-term Stored Lumber (Greater than 24 hours Storage)

Leachate from lumber which had been stored for greater than 24 hours on the mill yard was sampled using the same technique as that for freshly treated lumber. A drainage basin of known dimension was selected. Lumber within this basin was examined for production date to determine stacks which had shortest, medium and longest storage times since treatment. A 3.0 L heat treated, clear pyrex glass baking tray was placed in the space under each multiple stack and age group of lumber.(Plate #3,#4) Leachate dripping from the lumber was collected in the glass trays and then composited by pouring it into 1.0 L amber glass bottles.

The trays remained in the stack until the sampling sequence was completed or the lumber stacks were removed from the storage yard. This technique allowed measurement of TCMTB in the leachate dripping from the lumber, prior to dilution by precipitation on the mill yard.

3.4.1 **Leaching of TCMTB from Lumber Stored for Several Weeks**. Metal trays with polyethylene plastic liner were constructed to collect the leachate from two - 4.6 m x 1.2 m x 0.61 m (16 ft. x 4 ft x 2 ft) packages of planed lumber consisting of 48 boards of 0.20 m x 0.07 m x 4.6 m (8 in. x 3 in. x 16 ft.). A single hole in the plastic tray allowed leachate to be collected in a 10 L opaque glass jar. The lumber was continuously exposed to ambient precipitation over 11 days and subjected to 7 rainfall events. Subsamples were collected in heat treated amber glass jars and analysed for TCMTB and metabolites.(Plates #5,#6)

3.5 Sampling of Contaminated Rainwater Runoff

Lumber leachate which dripped from the lumber stacks migrates from the stack to a storm drain in the storage yard basin. Uncontaminated rainfall landing on the uncovered areas between stacks of lumber and vehicle alleys will add volume and dilute the leachate(Plate #7). The metal grating covering

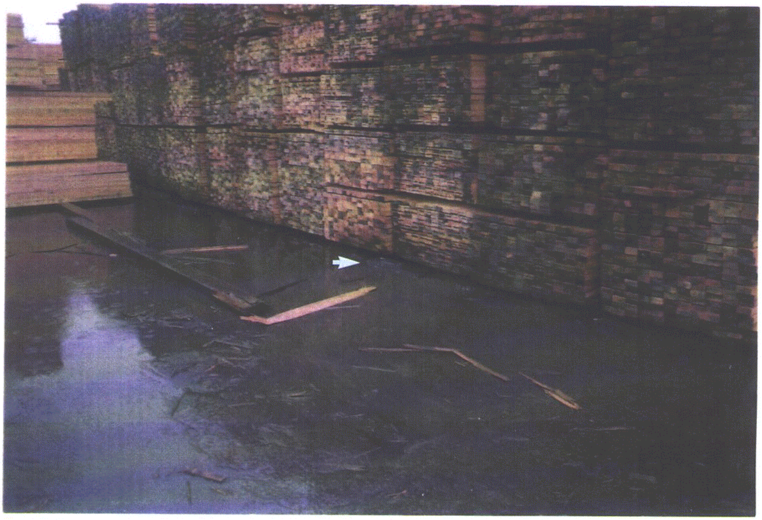


PLATE #3 Sampling of leachate from rough cut lumber. Arrow shows location of the sampling tray.

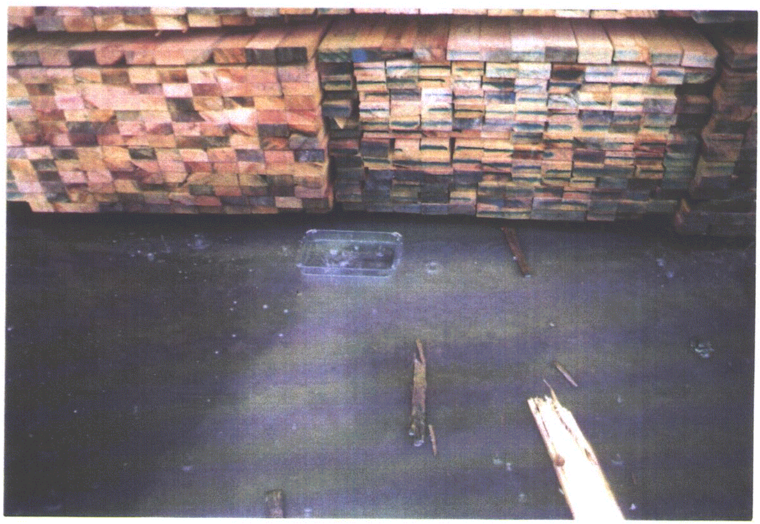


PLATE #4 Closeup of leachate sampling tray

PLATE #5 (left)
Steel support trays with plastic liners to collect leachate over multiple rainfall cycles

PLATE #6 (below)
Treated lumber placed on sampling trays to collect leachate over multiple rainfall cycles

PLATE #7 View of finished lumber on the long-term storage yard. Note the large open areas which are not covered by lumber

PLATE #8 Storm drain located in low area of the drainage basin was used as a sampling location. (See Arrow) the storm catchbasin was removed to facilitate sample collection(Plate #8). If necessary the runoff was directed to one flume by encircling the catchbasin with polyethylene covered sand bags. The yard runoff was then sampled at timed intervals by passing the mouth of the sample jar at an even rate across the flume of effluent until the desired sample volume was collected.

3.6 Sampling of Contaminated Storm Runoff from a Rough Cut Lumber Storage Yard

Runoff from the rough cut storage yard drained to a sump from which it was pumped into the Fraser River. The pump discharge pipes were sometimes covered by the high tide therefore samples were always collected from the sump.(Plate #9, #10) The samples were collected using a telescoping sample rod which was used to lower 1.0 L heat treated amber glass bottles to the bottom of the sump. The sample was collected by repeatedly raising and lowering a bottle from the bottom to the surface at a steady rate until the desired volume was collected. (Figure 3, Site A)

Runoff from the planed lumber storage yard to the Fraser River was sampled at the last manhole prior to discharge during flood tide periods when the outfall was covered by river water. These samples were collected using a telescoping pole which was used to lower 1.0 L heat treated amber glass bottles into the standpipe and positioning the mouth of the bottle at the standpipe discharge till the desired volume was collected. All other samples were collected at the point of discharge when the outfall was exposed above the tidal level of the river.(Plate #11, #12) The sample bottle was then hand held and moved back and forth through the discharge stream until the desired volume was collected.

3.7 Sampling of the Fraser River

Fraser River water samples were collected using a telescoping pole which was used to lower 1.0 L heat treated amber glass bottles to the desired depth and distance from the discharge point. The sample was collected by

PLATE #9 (1) Storm water collection sump which collects runoff from the sample site. (2) Pumps which discharge effluent to the Fraser River. (3) Discharge of effluent to the Fraser River

PLATE #10 Use of sampling rod to collect water samples at various depths. Arrows indicate sample sites R6, R7 and R8, from the rough cut lumber storage yard discharge

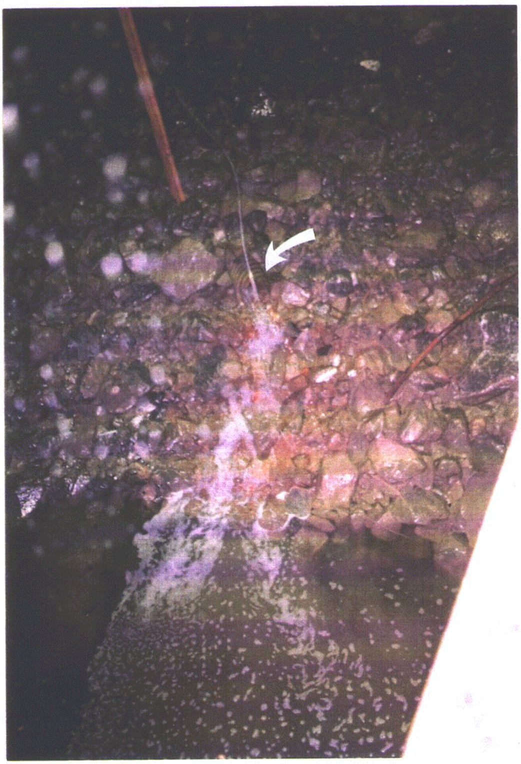


PLATE #11 (above)
Discharge of runoff from the planed lumber storage yard at flood tide. The green dye illustrates the early stages of plume formation

PLATE #12 (left)
Discharge of runoff from the planed lumber storage yard when the tide is below the level of the discharge pipe

positioning the bottle just below the surface in the center of the visible plume and then moving the bottle back and forth across the breadth of the plume until the desired volume was collected. Samples at lower depths were collected by using the telescoping pole to invert the bottle until the desired depth was reached. The bottle was then turned upright and moved back and forth across the plume at a steady rate until the escapement of air ceased. The bottle was then quickly withdrawn from the river in an inverted position which created a positive flow from the bottle but which would reduce the chance of sample contamination by passage through zones of higher or lower TCMTB concentration. Samples were collected at 5 m, 10 m, and 35 m from the discharge point(Plates #10 and #13).

3.8 Frequency of Sampling

3.8.1 Working Solution. A single grab sample of working solution was collected from the mix room tank. It was assumed that once the operating parameters of the mix tank metering system were set there would be little variation in the concentration over the sampling period. The working solution sample would not be affected by changes in rainfall.

3.8.2 Lumber Leachate. Lumber leachate dripping directly from the packages was sampled as a continuous composite ranging from one to three hours in duration, most commonly two hours.

3.8.3. Other Samples. At all other land locations the sampling was done as a single grab of 1.0 L at time = zero. Time zero was chosen as close to the start of the rainfall event as possible. Sampling during the first hour after time zero consisted of 0.5 L grabs every 15 minutes, composited as 1.0 L. The middle hours (between the first hour and the hour after the rainfall event ended) were sampled as 0.5 L grabs every 30 minutes composited as 1.0 L. This was continued until the rainfall ended. Sampling in the last hour after rainfall ended consisted of 0.5 L grabs every 15 minutes composited as 1.0 L.

In December 1989 sampling expanded to include both storm runoff samples and the river plume. Sampling frequency was changed to 0.5 L every

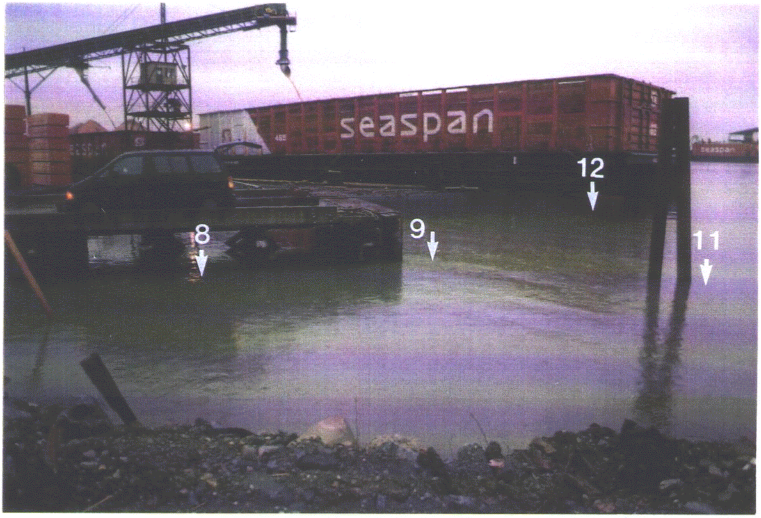


PLATE #13 Discharge of effluent from the planed lumber storage yard was monitored at sample locations P8, P9, P11, P12. Green dye shows the dimensions of the plume 15 minutes after the introduction of dye to the system

1/2 hour composited to 1.0 L at all locations. This was to accommodate the increased number of sample locations and the limit of 36 samples per storm event necessary to allow the laboratory to provide results within 24 hours of sampling.

3.9 Preservation and Storage of Samples

The amber glass sample bottles were stored in Coleman coolers and packed in crushed ice. No preservatives or pH buffers were added. Most samples were transported to the laboratory and extracted within 12 hours of sampling. For analytical procedures see Appendix #1.

3.10 Other Water Quality Parameters Measured

Tidally influenced river depth was recorded relative to the elevation of the outfall pipe at the planed lumber storage yard. Dissolved oxygen, conductivity, pH and temperature were measured using a Hydrolab Surveyor II multiprobe meter with sonde, 30 m cable, and meter unit.

3.11 Calibration of Monitoring Equipment

Temperature and depth were measured using the factory calibrated settings. All other parameters were calibrated in the laboratory under barometric conditions as close to the rainfall event as possible.

pH was measured by calibrating the Hydrolab at pH 4.0 and pH 7.0, or pH 7.0 and 10.0 using standard solutions. (The December 2, 1989 setting was calibrated at 4.0 and 7.0. Thereafter the calibration was at 7.0 and 10.0 as most readings in the Fraser River were slightly basic.)

Dissolved oxygen was calibrated using the saturated air technique as specified in the Hydrolab manual. This involves the measurement of oxygen in water saturated air corrected for temperature and barometric pressure.

Conductivity was calibrated using standard concentrations of KCl in deionized water. Standard solutions of 0.01, 0.05, and 0.1 molar Potassium chloride (KCl) with conductivities of 1.413, 6.668 and 12.9 millimhos/cm were used. Conductivities generally ranged from 1.0 to 12.7 mmho/cm in the river therefore the two lower standards were used most often to calibrate the equipment.

3.12 Measurement of Stormwater Discharge Rate and River Depth

The stormwater discharge from the planed lumber storage yard was measured at the end of the outfall. Flow measurements could only be taken when the tidal river level was below the level of outfall. (Figure #3, site P7) A calibrated bucket of 10.5 liters total volume was used. The time to fill the bucket was recorded for three times during each sample collection period. The average time for the total flow to fill the bucket was recorded. During times of high intensity rainfall the flow rate could exceed the ability to measure it and the discharge rate was recorded as greater than 10.5 L/s.

The river depth was measured in 1.0 m increments from the river bottom. The other physical parameters were measured at these locations to determine if there was movement of a salt water wedge or change in pH with depth and time.

4.0 MONITORING RESULTS

Grab samples of leachates and effluents were collected on November 3 and November 27, December 4, 1989 and January 3, 1990. Composite samples over extended periods of rainfall were collected on December 2, December 7, 1989 and January 9, 1990. A controlled leaching test of treated lumber was carried out from January 10 to January 21, 1990.

4.1 Concentration of TCMTB in the Working Solution

The concentration of TCMTB in the working solution of the planer mill mixtank ranged from 2,340,000 ppb (0.23%) on November 11, 1989 to 10,100,000 (1.01 %) on December 7, 1989. The average for four samples was 4,510,000 ppb (0.45%) TCMTB. The concentration of TCMTB in the rough cut lumber mixroom was 3,680,000 ppb (0.368 %) on December 2, 1989.

TABLE 1: THE CONCENTRATION OF TCMTB, MBT, BT AND MMBT IN THE MIXROOM WORKING SOLUTION (ppb)

TCMTB	MBT	BT	MMBT
Rough cut mixroom			
Nov. 03 - 2,340,000	15,800	5,200	2,240
Planed lumber mixroom			
Dec. 02 - 3,680,000	<625	<500	10,200
Dec. 07 - 10,100,000	<62,000	<50,000	9,280
Jan. 03 - 2,230,000	<312,000	<100,000	8,340
Jan. 09 - 3,370,000	<25,000	<20,000	16,700
Average = 4,510,000			9352+/-5,155

Note: High concentrations of TCMTB require multiple dilutions which increase the detection limits for MBT and BT

4.2 Surface Application Rate of TCMTB

Monthly quality control tests are conducted for the mill by a contract laboratory. The surface application rate of TCMTB averaged 61.3 ug/cm2 and ranged from 43.0 to 89.7 mg/cm^2 in the monthly tests conducted from July to Nov. 1988. Test results from the mills quality control program were not available for December and January.

4.3 Concentration of TCMTB, MBT, BT, MMBT in Leachate from Freshly Treated Lumber

Leachate was collected from treated lumber which had from 1 to 11 minutes covered storage prior to exposure to rainfall. This was considered freshly treated lumber. The concentration of TCMTB in the leachate of freshly treated lumber ranged from 41,500 ppb to 168,000 ppb with an average of 88,875 +/- 56,490 ppb for four samples collected as two hour composites each.(Table 2) Plates #14, #15 and #16 show that TCMTB was readily washed from the treated lumber. The discolouration shown in the photographs is due to the presence of TCMTB. (Wax was not used in the formulation).

TABLE 2: CONCENTRATION OF TCMTB, MBT, BT AND MMBT IN THE LEACHATE AND YARD RUNOFF FROM FRESHLY TREATED LUMBER (ppb)

Leachate from Freshly Treated Lumber*				
DATE	TCMTB	MBT	BT	MMBT
Nov. 03	89,800	<50	<20	130
Dec. 07	168,000	<25	<20	3,550
Dec. 07	56,200	<25	<20	229
Dec. 07	41,500	<25	<20	156
Mean	= 88,875 ± 56,490	<25	<20	1,016
Freshly Treated Lumber Storage Yard Runoff				
Nov. 03	3,300	1,040	<20	24
Dec. 07	11,800	1,270	<20	102
Mean	= 7,550 ± 6,010	1,155	<20	63

* No covered storage after treatment

PLATE #14 Leachate from lumber which was treated in a high pressure spray booth. The concentration of TCMTB ranged from 41,500 ppb to 168,000 ppb

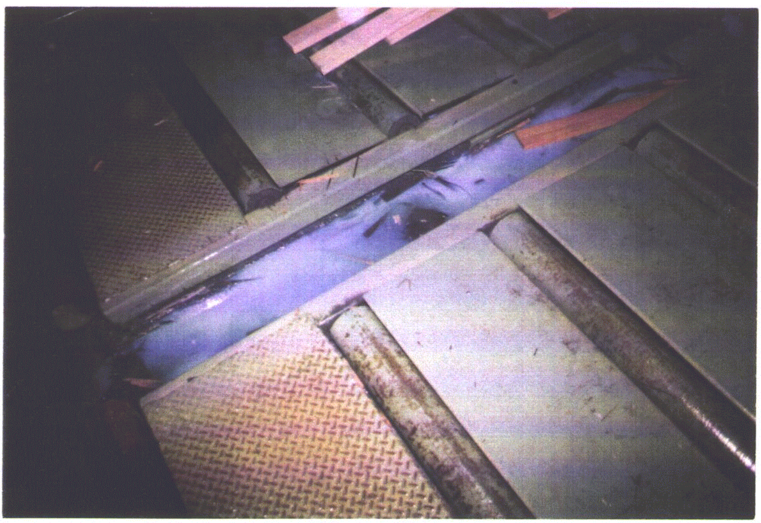


PLATE #15 Leachate from the freshly treated lumber collects on the storage yard

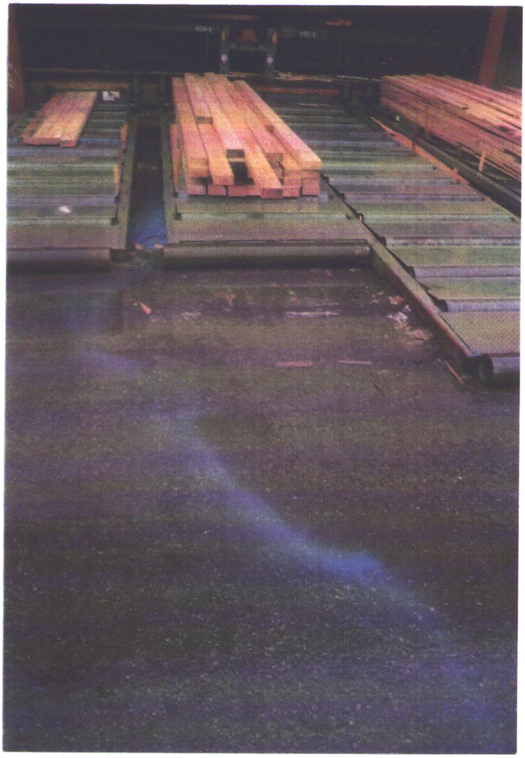


PLATE #16
Leachate becomes diluted as it migrates across the mill yard. Discharge to the sewer ranged from 3300 ppb to 11,800 ppb

The concentration of TCMTB in two samples was 3,300 ppb (R2, Figure 3) and 11,800 ppb (R1, Figure 3) in yard runoff draining from the freshly treated lumber storage area. Settling of the yard caused the runoff to bypass the closest storm drain R1. The sample containing 11,800 ppb was collected at the bypass to the R1 storm drain. The sample of 3,300 ppb was collected at the R2 storm drain which was twice the distance from the lumber packages normally associated with this area. The average concentration discharging to the drain was 7,550 ppb.(Table 2) These results illustrate the typical order of magnitude of the dilution effect, which was found to occur between the leachate dripping from the lumber (88,875 ppb) and the runoff entering the nearest storm drain (7,550 ppb).

The leachate from the freshly treated lumber did not have any detectable MBT whereas the yard runoff from the area did. The difference is likely due to the residual TCMTB left on the yard undergoing decomposition. The possible mechanism is discussed in Chapter 6.

4.4 TCMTB in Leachate from Lumber with Greater Than 24 Hrs. Storage

The study mill did not have covered storage therefore extended drying after treatment occurred only when there was no precipitation. Leachate dripping from treated lumber which had greater than 24 hours drying time after treatment was collected from three sources. Two random composites of leachate were collected from rough cut lumber and six composites were collected from planed lumber in the storage yards. Seven controlled composites were collected from planed lumber which was isolated from the production line immediately after treatment.

The rough cut leachate samples consisted of two composite samples collected on November 3 and December 2, 1989.(Table #3) The November 3 sample was from a single stack of five packages which had been stored on the yard for an undetermined length of time. (It is suspected that much of the TCMTB had been leached or degraded by ultraviolet light prior to the sampling as mold was already starting to grow on the lumber surface.) This sample had a concentration of only 229 ppb TCMTB. (See section 6)

A second sample was collected on December 2, 1989 by compositing leachate from three separate stacks of five packages over a three hour period. This sample contained 3700 ppb TCMTB which was more characteristic of other samples collected from planed lumber.

Six random composites were collected on four different storm events from stacks of planed lumber which were five packages high.(Table #3) TCMTB concentrations ranged from 4,510 to 9,080 ppb with an average value of 5,865 +/- 1,946 ppb. (See Figures 5-2, 6-4, 7-2)

TABLE 3 TCMTB, MBT, BT AND MMBT IN LEACHATE FROM LUMBER WITH GREATER THAN 24 HOURS COVERED STORAGE AFTER TREATMENT (ppb)

DATE	TCMTB	MBT	BT	MMBT
Rough Cut				
Nov. 3*	229	4740	<20	33
Dec. 2	3700	<625	<500	143
Planed Cut				
Nov. 3	5390	680	270	5
Dec. 4	4510	<25	<20	12
Dec. 7**	8340,8780,9080	all <25	all <20	20,14,13
Jan. 9	4830	<25	<20	17
Mean	5433			

* This sample was from lumber which had been in the yard for longer than the normal storage period. Fungus was visible on the lumber surface, therefore this sample was excluded in the calculation of the mean.

** These three numbers were averaged prior to calculating the mean.

Two packages of lumber were isolated from the production line and placed on a leachate sampling tray.(Plate #6) Composite samples of leachate were collected during seven rainfall events over 11 days.(Table #4) Figure #4-1 shows a relatively constant decrease in concentration from 9,045 ppb to 2590 ppb TCMTB over this period. The cumulative rainfall was greater than 24.4 mm for the seven events (note that rainfall was not recorded on the last day). The average concentration of all these samples was 4,715 +/- 2,572 ppb.

TABLE 4 TCMTB, MBT, BT AND MMBT IN LEACHATE FROM PLANED LUMBER WITH GREATER THAN 24 HOURS COVERED STORAGE OVER 7 LEACHING/DRYING CYCLES (ppb)

JANUARY	11	13	14	15	16	21	22	AVERAGE
TCMTB	9045	6300	5890	2660	4690	1830	2590	4715 +/- 2572
MBT	<25	<25	<25	<25	<25	<25	<25	
BT	<20	<20	<20	<20	<20	<20	<20	
MMBT	11	<5	<5	<5	<5	<5	<5	

The mean concentration of TCMTB in leachate from freshly treated lumber which had no covered storage time after treatment was 88,875 ppb. (Table 2) The concentration of TCMTB in leachate from lumber which has had at least 24 hours to dry after treatment is an order of magnitude less ranging from 4715 to 5433 ppb.(Table 3 and 4) It was expected that rough cut lumber would leach at a concentration higher than planed lumber due to the higher surface area however this was not observed. Planed lumber is only dimensioned on two sides. This results in the two unplaned sides from this mill of each board receiving double treatment which may explain the higher concentration in the leachate from planed lumber.

4.5 Discharge of TCMTB Contaminated Rainwater Runoff From a Rough Cut Lumber Yard

Contaminated rainwater runoff discharging into the Fraser River from the rough cut lumber yard was sampled on three different rainfall events. The average concentration of TCMTB in the seven samples collected, was 229 ppb with a range of 68 ppb to 479 ppb. The yard was subject to flooding caused by the overflow of street runoff from a city storm sewer. It is suspected that this caused significant dilution in three of the seven samples and the actual average concentration is believed to be closer to 300 ppb.(Table 5)

Figure #4: The Concentration of TCMTB in Leachate from Treated Lumber over Multiple Leaching Cycles

Figure 4-1: Concentration of TCMTB in Leachate over Multiple Cycles

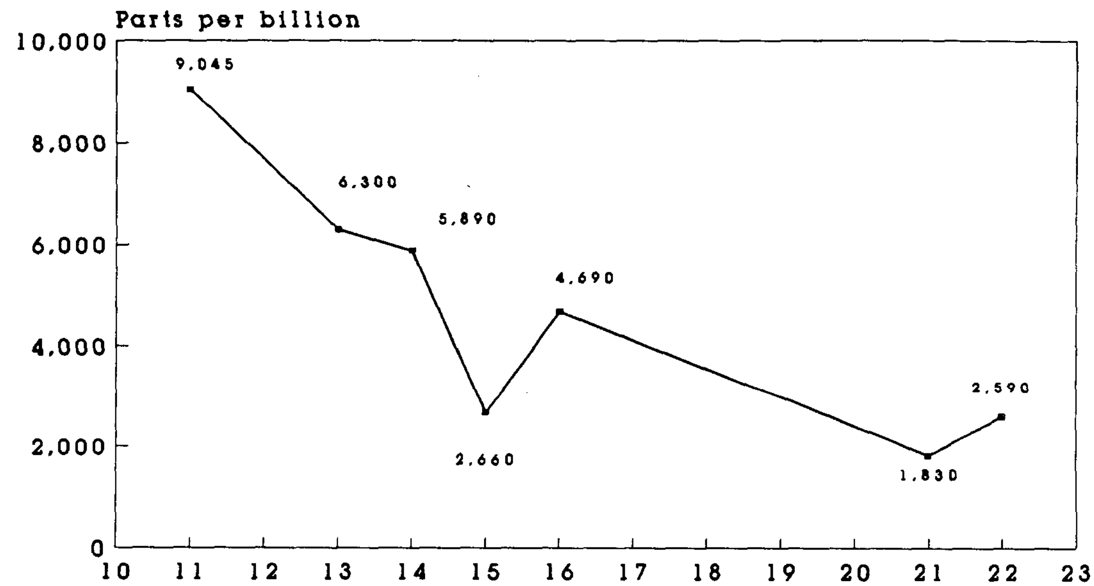


Figure 4-2: Cummulative Rainfall per Day

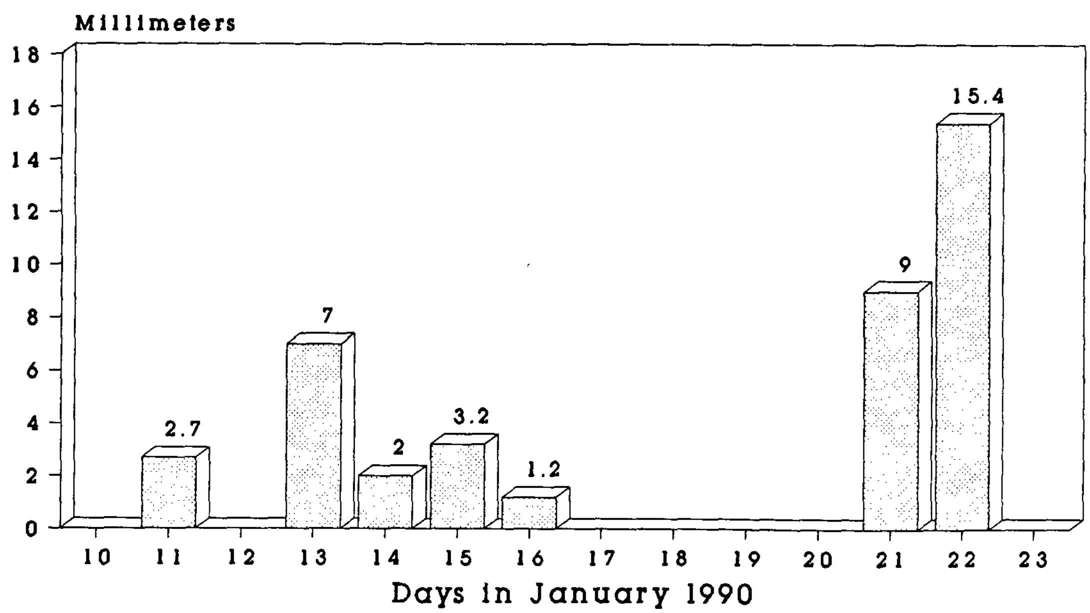


TABLE 5 CONCENTRATIONS OF TCMTB, MBT, BT AND MMBT IN RUNOFF WATER FROM A ROUGH CUT TREATED LUMBER YARD (ppb)

	TCMTB	MBT	BT	MMBT
Nov 3	68	1220	<20	12
Nov 8,9	479	304	<20	12
Nov 27	187	<25	<20	31
Dec 2	128, 263, 182, 302	all <25	all <20	75,33,38,31
Average	229 ± 135	<393 ± 566	<20	33 ± 21

4.5.1 <u>Concentration of TCMTB in the Fraser River Adjacent to the Rough Cut Lumber Storage Yard</u>. The contaminated runoff from the rough cut lumber yard was discharged into the Fraser River from a sump via float activated pumps. The frequency and duration of the discharges were directly dependent on the intensity of the rainfall and the volume of runoff. During the discharge periods, samples of river water were collected in the center of the plume at distances of five and ten meters from the outfall. The surface concentration at five meters from the outfall was 74 +/- 23 ppb TCMTB. At ten meters from the outfall the concentration averaged 49 +/- 17 ppb.(Table 6, Figures 5-3, 5-4)

TABLE 6 CONCENTRATIONS OF TCMTB, MBT, BT, AND MMBT IN THE FRASER RIVER ADJACENT TO THE ROUGH CUT STORAGE YARD EFFLUENT DISCHARGE (ppb)

	TCMTB	MBT	BT	MMBT
5.0 m from discharge at the surface Dec. 2	65,98,89,46	all <25	<20	10,10,9,5
Average	74 ± 23			8.5 ± 2.4
10.0 m from the discharge at the surface Nov. 3	<8.0			
Dec. 2	64,57,50,25	all <25	all <20	10,6,5,<5
Average	49 ± 17			6.5 ± 2.4

4.5.2 Discharge of TCMTB Contaminated Runoff from an Uncovered, Planed Lumber Yard

The finished lumber storage yard contained two types of lumber packages. Forty-five percent of the lumber was planed, strapped, endsealed and wrapped in plastic on the top and two ends. The remaining 55% did not have the plastic wrapping. The yard was isolated from other sources of dilution. Effluent was monitored during five separate storm events and 17 samples were collected. The average concentration of TCMTB was 337 +/- 117 ppb. and ranged from 185 to 533 ppb. (Table 7, see also Figures 6-5, 7-3)

TABLE 7 CONCENTRATIONS OF TCMTB, MBT, BT, AND MMBT IN CONTAMINATED RUNOFF FROM AN UNCOVERED, PLANED LUMBER STORAGE YARD (ppb)

	TCMTB	MBT	BT	MMBT
Nov. 3	355	<50	<20	<5
Nov. 27	35*			
Dec. 4	246, 205	all <25	all <20	16,15
Dec. 7	196, 185, 219, 305			
	307, 513, 533, 489	all <25	all <20	all <5
Jan. 3	348, 260	all <25	all <20	all <5
Jan. 9	295, 350, 484, 442	all <25	all <20	27,24,20,22
Average	337 $\pm$ 117 ppb	<25	<25	

* (excluded as it was not raining on this day)

Due to the large number of operating and environmental variables which occur at the sawmill, it is difficult to co-relate ratios of decomposition products. When TCMTB is subjected to weathering, decomposition products BT, MBT and MMBT were present in the samples. These decomposition products were found in leachate from treated wood which had been stored in the yard for long periods.

4.5.3 Concentrations of TCMTB in the Fraser River Adjacent to an Uncovered, Planed Lumber Storage Yard

Samples of Fraser River water were collected at 5.0 m, 10.0 and 35.0 meters from the outfall. The following averages are a composite of all data collected which includes periods when the discharge was above and below the influence of the river.(Table 8, see also Figures 6-6, 6-7, 7-4, 7-5)

At 5.0 meters from the outfall the average concentration at the surface was 65 ± 27 ppb, 18 ± 13 ppb one meter below, and 3 ± 3 ppb two meters below the surface.

At 10.0 meters from the outfall the average concentration at the surface was 29 ± 20 ppb, 2.2 ± 2.2 ppb one half meter below and <1.0 ppb at one meter below the surface.

At 35.0 meters from the outfall the concentrations of TCMTB was less than 1.0 ppb at all depths. The co-efficient of variation was very high for all sampling points. This is due to the variations caused by rain, tide and river flow.

TABLE 8 CONCENTRATION OF TCMTB, IN THE FRASER RIVER ADJACENT TO A FINISHED LUMBER STORAGE YARD (ppb)

LOCATION	NUMBER OF SAMPLES	MEAN +/- Sd*	CO-EFFICIENT OF VARIATION	RANGE
5.0 m from outfall:				
surface	14	65 +/- 27	42%	19 - 110
1.0 meter below surface	6	18 +/- 13	72%	6 - 38
2.0 meter below surface	5	3 +/- 3	100%	
10.0 m from the outfall				
surface	14	29 +/- 20	69%	<1.0 to 72
0.5 meter below surface	4	2.2 +/- 2.5	100%	
1.0 meter below surface	3	<1.0		
35.0 m from the outfall				
surface	4	<1.0		
0.5 meter below surface	4	<1.0		
1.0 meter below surface	1	<1.0		

* Sd = Standard deviation

5.0 CORRELATION OF TCMTB CONCENTRATIONS IN TREATED LUMBER STORAGE YARD EFFLUENT AND FRASER RIVER WATER DURING RAINFALL EVENTS

In Section 4 data was presented without taking into account the conditions which affect the concentrations of TCMTB in the leachate and the runoff. As such they report the average ranges which could be expected to be found at the various locations on any given rainfall event. This section will deal with three specific rainfall events and correlate the various parameters in order to provide a visual profile of the factors which can influence the concentration of TCMTB in various effluent streams.

5.1 Rainfall and TCMTB Concentration Profiles in Effluent from a Rough Cut Lumber Storage Yard

On December 2, 1989 the rough lumber storage yard was monitored during a storm which had a total accumulation of 10.7 mm of rain. (Figure 5) Accumulated rainfall was 6.9 mm prior to the start of sampling and therefore only the end of the storm event was monitored.(Figure 5-1) Two other complicating factors were that:

- a city storm sewer which passes under the property had backed up due to the high tide and was overflowing to the lumber storage yard

- a mill maintenance crew had shut off the power for the pumps which serviced the sump draining the rough cut yard

- time of the power shut off was not recorded by the maintenance crew

These factors caused considerable flooding on the yard and dilution of the runoff. The pumps were reactivated and by 11:00 and the surface flooding of the yard ceased. It is not known how much dilution was caused by the continual overflow of the city sewer to the rough cut lumber storage yard or when this dilution effect ended.

Figure 5: Rainfall vs Concentration of TCMTB in Runoff, Effluent and Fraser River Water

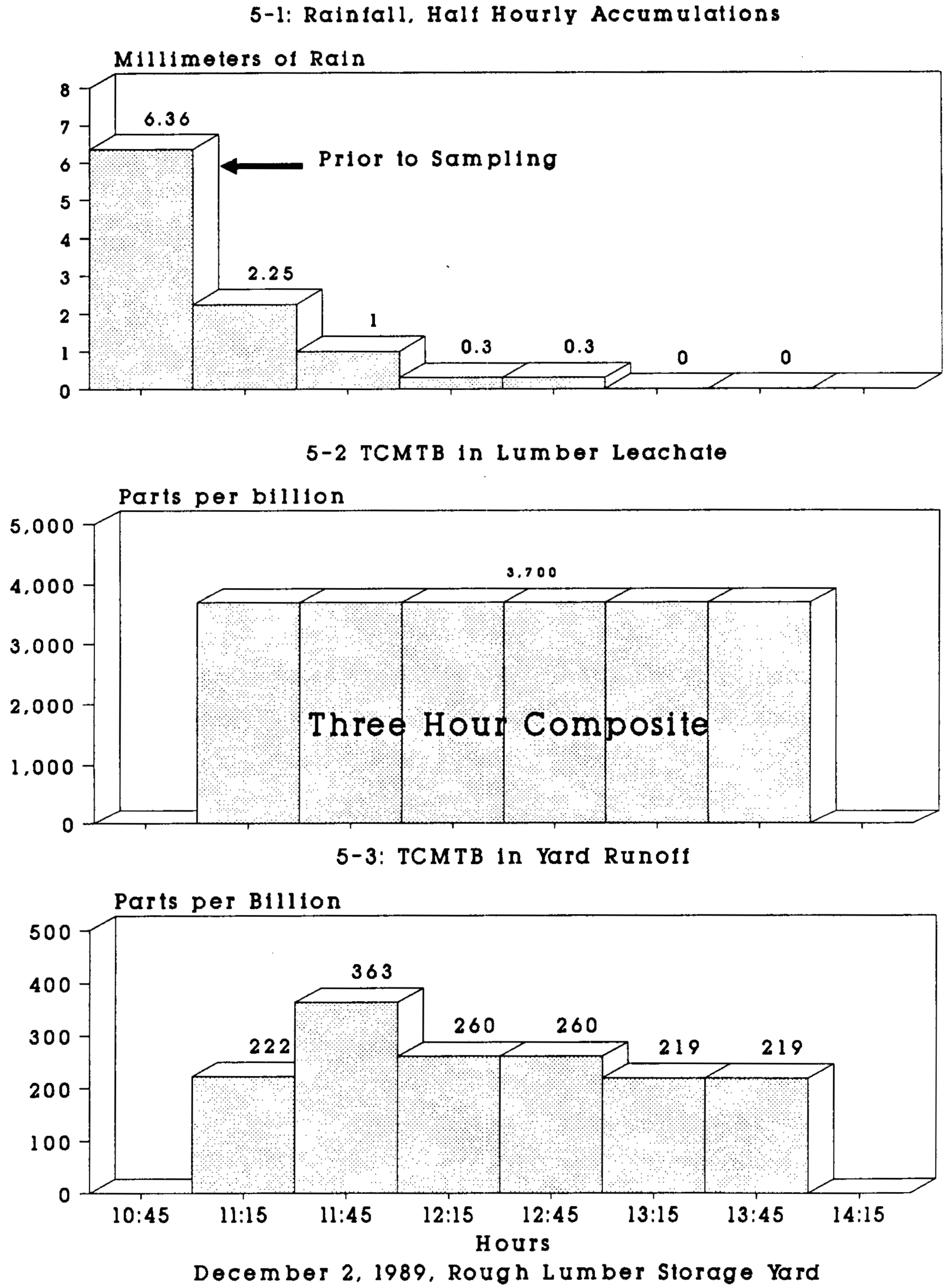


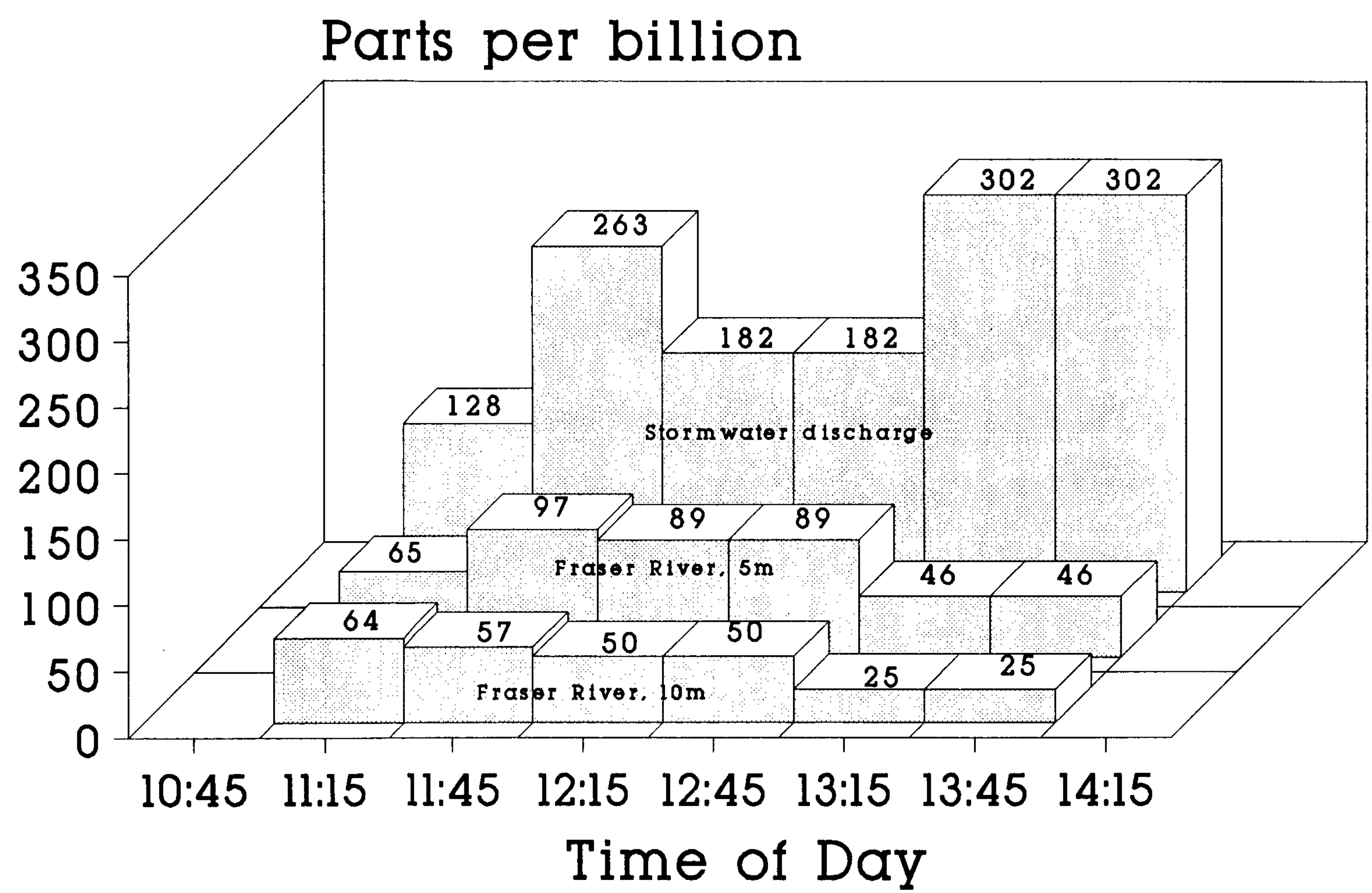
Figure 5-2, shows that the leachate dripping directly from the lumber contained 3700 ppb TCMTB. This was an order of magnitude (14 times) greater than the concentration of TCMTB in the yard runoff. The average value of 3700 ppb is 247 times the freshwater 96 h LC50 of 15 ppb for chinook salmon in fresh water.

Figure 5-3, shows that the concentration of TCMTB in the runoff which migrated from the lumber packages to the entrance to the nearest stormdrain. The concentration profile did not show the characteristic peaking of the concentration which occurs when the rainfall stops and the yard runoff is predominantly undiluted leachate. These samples were collected at a point which was not affected by the city sewer overflow and therefore dilution by outside sources would not account for the stable concentration profile. The average value of 262 ppb is 18 times the freshwater 96 h LC50 for salmon and 18 times the allowable limit of 15 ppb as specified in the B.C. Waste Management Act Regulations.(8)

Figure 5-4, shows the concentration of TCMTB in stormwater which discharges from the rough lumber storage yard. The concentration of TCMTB in the surface water of the Fraser River, 5 m and 10 m from the discharge is also shown. The discharge to the Fraser River averaged 219 $\pm$ 78 ppb TCMTB, the 5m concentration averaged 74 $\pm$ 23 ppb TCMTB. The 10 m concentration averaged 49 $\pm$ 17 ppb TCMTB. This is 15, 5, and 3 times the freshwater 96 h LC50 for salmon.

Sampling in the Fraser River was complicated by three factors. The pumps which service the sump were activated by a float switch that creates an intermittent discharge with a frequency directly proportional to the intensity of the rainfall. To determine the worst case scenario, sampling was conducted during the discharge of the pumps(Plate #9, #10). A floating log deck 2 m from the discharge restricted the flow of the plume along the shoreline rather than outward into the river(Plate #10). The sampling was conducted on a Saturday when the logboom boats were not operating in the area

5-4: TCMTB in Final Stormwater Discharge and Surface water, 5m,10 m in Fraser R.



Dec.2, 1989, Rough Lumber Storage Yard.

therefore there was no turbulence which would disperse the effluent. The tide was at the peak and ebbing so that river velocity was low which would also reduce dispersion. The concentration in the river, 5 m from the discharge peaked at 97 ppb and declined to 46 ppb after the storm ended. The decline of the concentration of TCMTB in the river to 46 ppb does not reflect the increase to 302 ppb of the discharge. This decline is due to the tapering off of the rainfall which decreases the frequency of discharge from the pump. By 13:30 the dilution by the river water was the predominant influence on concentration. The average concentration of TCMTB 5 m into the river water was 4.9 times the freshwater 96 h LC50 for salmon and remained above the LC50 for the three hour monitoring period.

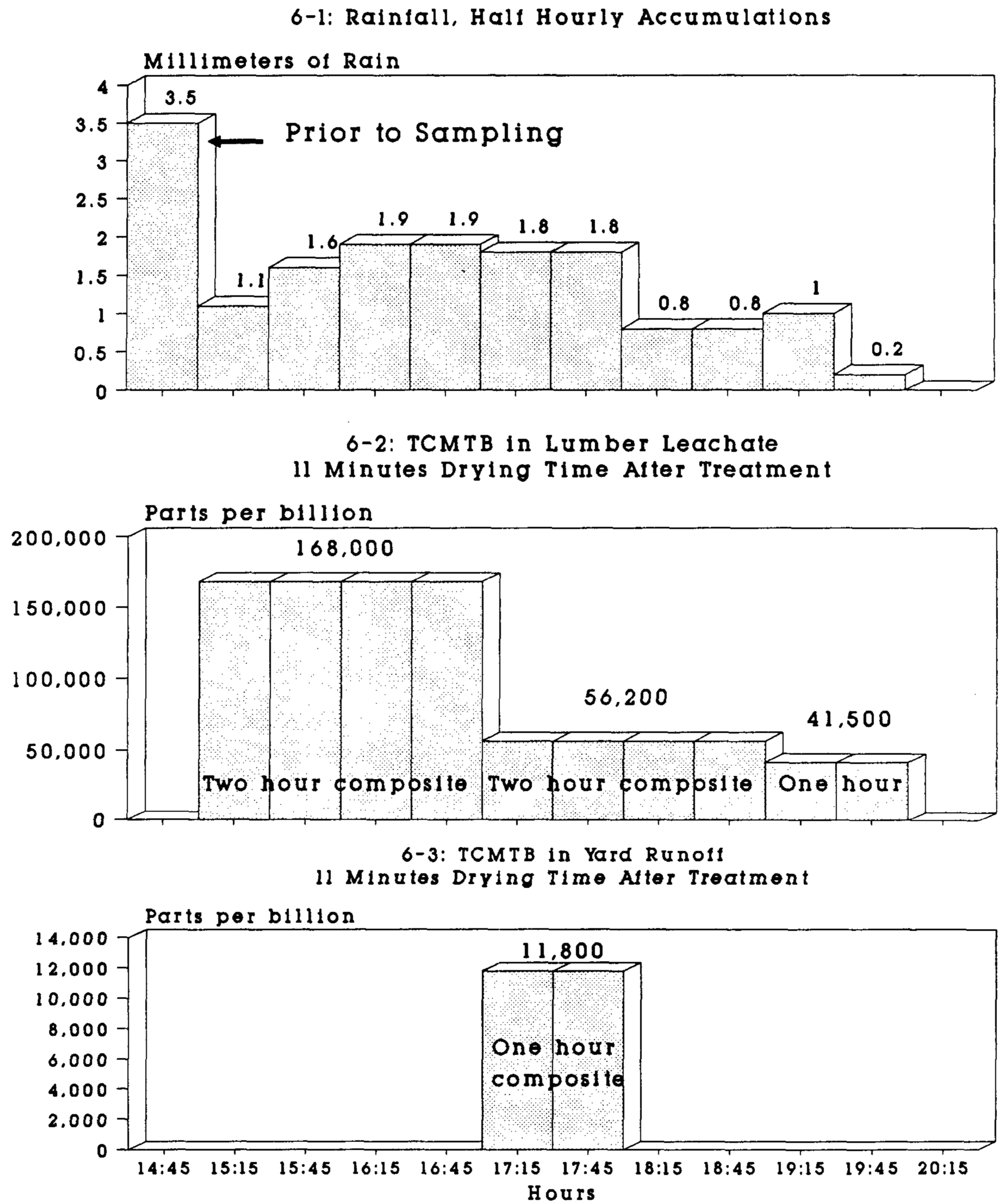
The concentration in the river, 10 m from the discharge decreased from 64 ppb to 25 ppb TCMTB, directly proportional to the decreasing rainfall profile. This indicated that the peak in concentration of TCMTB had already occurred and that the decreasing discharge volume caused the front of the contaminated plume to retreat towards the point of discharge. It is significant to note that the average concentration of TCMTB was always above the 96 h LC50 for the three hour monitoring period. It is not known how far the 15 ppb isopleth extended beyond the 10 meters sampling point.

5.2 Rainfall, TCMTB Concentration and Effluent Discharge Volume Profiles from a Planed Lumber Storage Yard

Figure 6 shows the rainfall accumulation at the planed lumber storage yard and TCMTB concentrations and runoff volumes for a storm monitored on Dec. 7, 1989. The drainage area of the catchment basin was 4.4 ha (10.8 acres) and contained an estimated 3,400,000 boardfeet of lumber. Lumber packages with the top and two ends wrapped in polyethylene plastic consisted of 43% of the total volume on the yard. Unwrapped lumber comprised of 57% of the total volume.(Plates #7,#8)

Figure 6-1 shows accumulation of rainfall prior to the start of sampling was 3.53 mm. Rainfall was continuous throughout the five hour

Figure 6: Rainfall vs Concentration of TCMTB in Runoff, Effluent and Fraser River Water



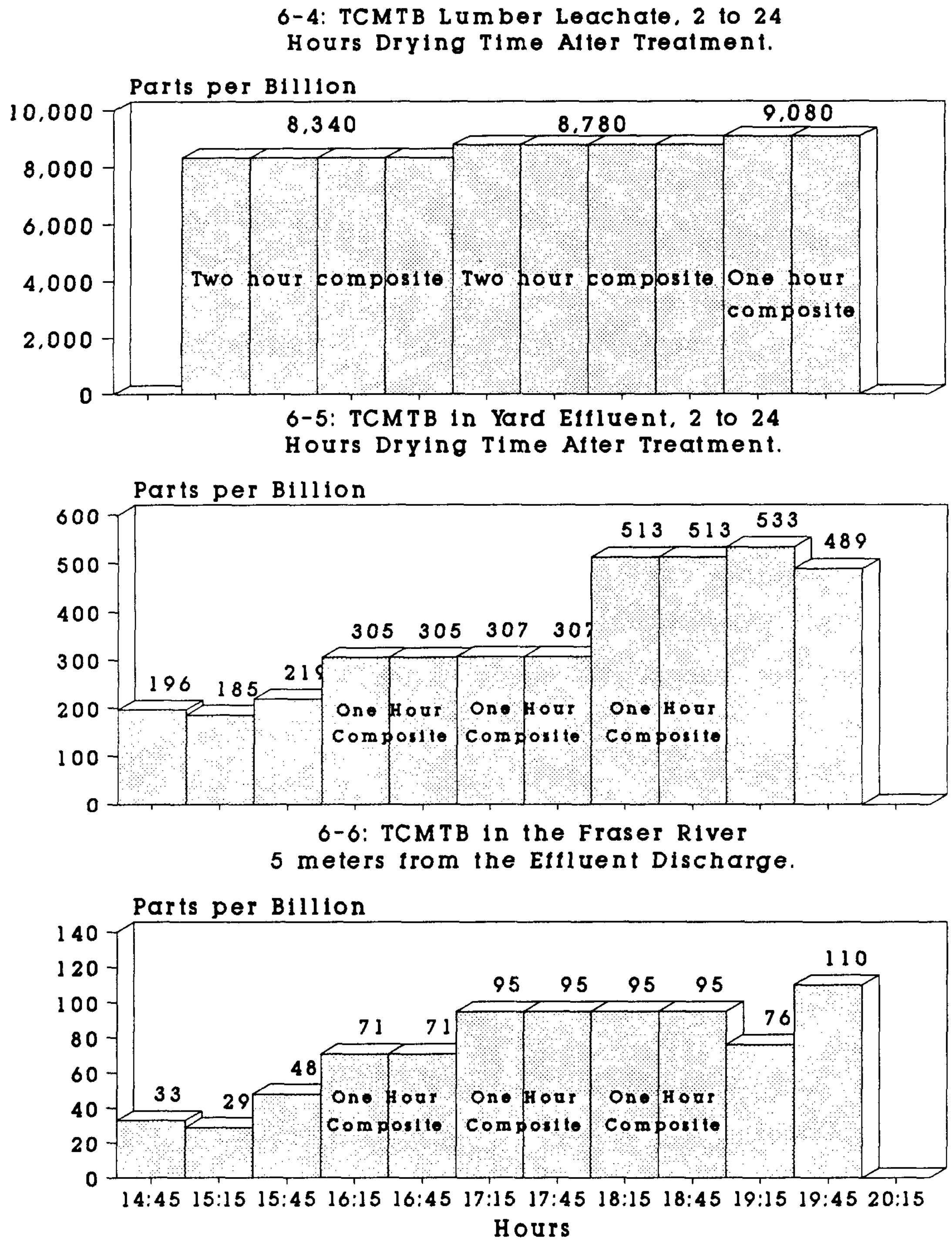
December 7, 1989, Planed Lumber Storage Yard monitoring period with a peak value of 1.9 mm per 30 minutes. After sampling was discontinued an additional 3.3 mm rainfall was recorded for a total accumulation of 19.7 mm.

Figure 6-2 shows the concentration in the leachate from freshly planed lumber which was being treated with a working concentration of 10,100,000 ppb (1.1%) TCMTB. This freshly treated lumber was observed to leach at such a rate that the yard was discoloured with the chemical. (Plate #14,#15,#16) The concentrations of TCMTB ranged from 168,000 to 41,500 ppb. This decline may be attributed to a decrease in the intensity of the rainfall and the quantity of chemical remaining on the surface of the treated lumber. The average concentration in the leachate was 88,567 +/- 69,182 ppb.

Figure 6-3, is of a single one hour composite sample of yard runoff collected at the storm drain which serviced the storage of freshly treated lumber. This effluent had a concentration of 11,800 ppb TCMTB. Settling of the soil under the pavement had caused the runoff to bypass the closest storm drain however a depression allowed the collection of the effluent at a point where it would normally have discharged to the drain. The concentration of 11,800 ppb is an order of magnitude lower than the leachate dripping from the freshly treated wood and is due to dilution by rainfall as the leachate migrates across the yard to the storm drain.

Figure 6-4, shows the concentration TCMTB in composite samples of leachate dripping directly from three stacks of lumber which had at least two to 24 hours drying time after treatment. Concentrations of TCMTB ranged from 8,340 to 9,080 ppb with an average value of 8,733 +/- 372 ppb. In Figure 6-4 the concentrations appear to the increasing. The increases are well within the normal variability of these samples however a reduced dilution effect due to the decreasing rainfall intensity is likely a cause for the rise in concentration. Once the lumber packages have been soaked they will continue to drip leachate well after the rainfall has ended and thus a rise in concentration will normally occur at the end of the storm dilution decreases.

Figure 6: Rainfall vs Concentration of TCMTB Continued



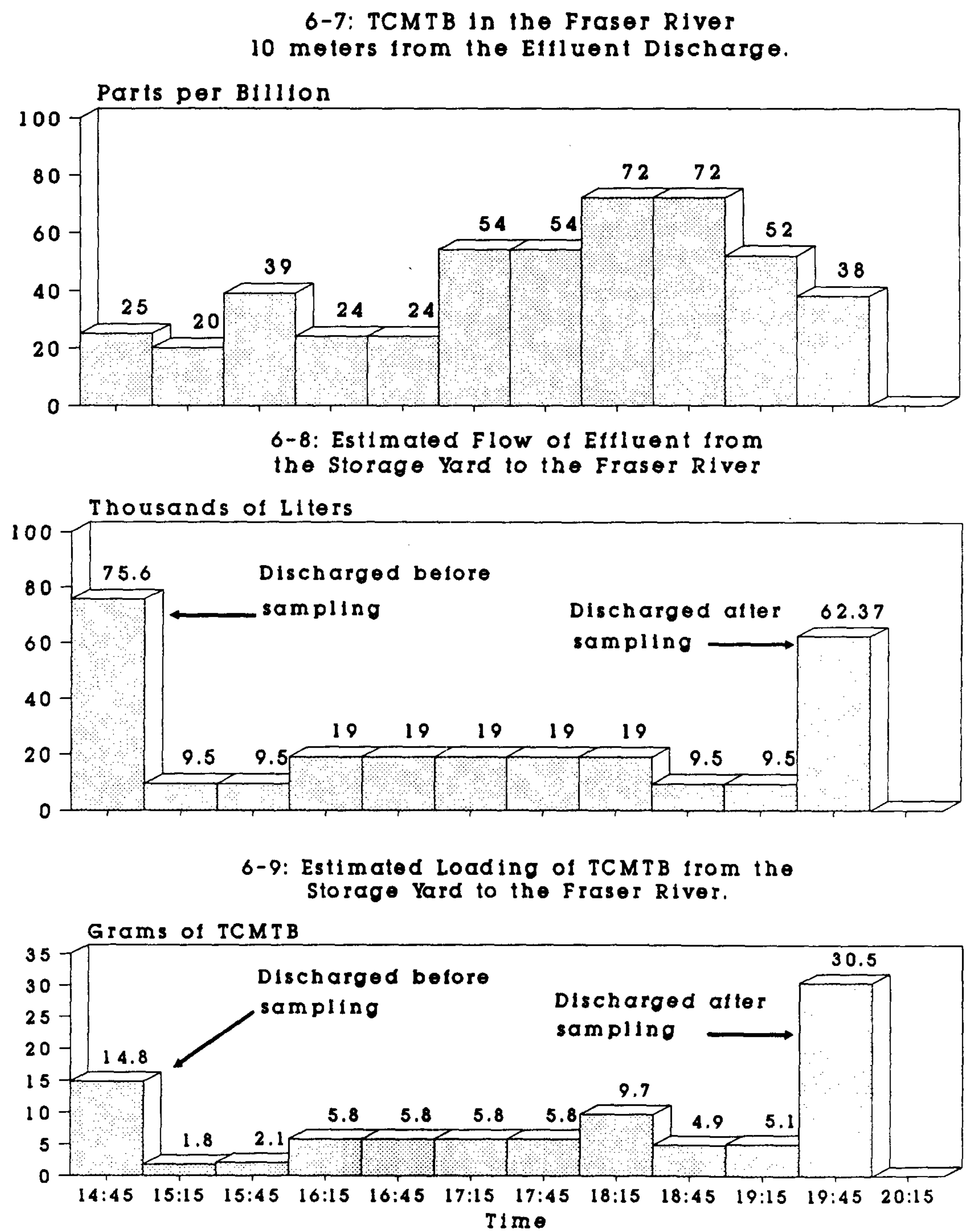
December 7/89, Planed Lumber Storage Yard Cont.

Figure 6-5 shows TCMTB concentrations in composite samples of effluent discharging from the planed lumber storage yard. The concentration of TCMTB in the effluent was inversely proportional to the intensity of the rainfall and ranged from 185 ppb to 533 ppb with an average value of 348 +/- 140 ppb. This is approximately an order of magnitude lower than the concentration in the leachate dripping from the lumber (Figure 6-4) and the same order of magnitude as measured in the discharge from the rough cut lumber storage yard.(Figure 5-3).

Figure 6-6 shows TCMTB concentrations in Fraser River surface water samples collected in the center of a plume 5 m from the discharge of runoff from the planed lumber storage yard. During this sample period, the tidal height of the river was at or below the level of the culvert. The velocity of the discharge pipe created a surface plume which was visible up to 30 meters from shore. (Plate #11, 12, 13) The concentration of TCMTB was found to range from 29 ppb to 110 ppb with an average of 72 +/- 29 ppb. The concentration profile was directly proportional to that in the effluent with a 4.8 times dilution. It is significant to note that the LC50 of 15 ppb was exceeded for the entire five hour monitoring period. The last three hours of monitoring showed that the concentration averaged 94 ppb TCMTB which indicates that the acutely toxic zone extended well beyond five meters.

Figure 6-7 shows TCMTB concentrations in Fraser River water samples which were collected in the plume 10 m from the discharge of runoff from the planed lumber storage yard. The concentration was found to range from 20 ppb to 72 ppb with an average of 42 +/- 19 ppb which is an 8.3 fold dilution of the effluent concentration. The concentration profile was directly proportional to the effluent profile except that it shows a decline from 72 to 38 ppb near the end of the storm event. This is due to the decreasing flow of the discharge which is causing the plume to shrink back to the source and results in the corresponding dilution effect. It is significant to note that the LC50 of 15 ppb was exceeded for the entire five hour monitoring period.

Figure 6: Rainfall vs Concentration of TCMTB Continued



December 7, 1989, Planed Lumber Storage Yard

The visible plume extended approximately 20 to 30 meters from the shore and swept in an arc from shore to shore depending on the direction of river flow which was co-current with ebb and flood of the tide. The distance from the shoreline of the 15 ppb isopleth of the plume is not known but could extend as far as 20 to 30 meters. The discharge temperature of the water was 1.0 to 2.0 Celcius degrees warmer than the river and the plume was believed to lie on the surface of the river to a depth of less than 1.0 meter. This is an extremely sensitive area as juvenile salmonids rear in surface and nearshore waters.

Figure 6-8 provides an estimate of the volume of runoff discharged from the culvert.

Rainfall was collected continuously and recorded at each sampling. Total volume of effluent per millimeter of rain could be estimated by dividing discharge rate by rainfall accumulation. Losses attributable to evaporation, absorption by lumber stacks and to ground infiltration were estimated at 18,900 L/mm based on measured flowrate and rainfall accumulation. A total of 19.5 mm of rainfall which fell during the storm would be estimated to generate 366,200 L of effluent. When the calculation is based on total rainfall and surface area and ignores the losses mentioned above, the volume discharged is 831,000 L. The actual volume is thus between 366,200 L and 831,000 L.

Figure 6-9 estimates the quantity of TCMTB discharged to the Fraser River from the planed lumber storage yard ranging from 92 grams to 253 grams depending on how the runoff volume is calculated.

The planed lumber storage yard comprised approximately 63% of the total storage area therefore the total estimated volume discharged from the yard during this storm was estimated at 585,400 L with an average concentration of 305 ppb TCMTB. This would deposit an estimated 146 to 180 grams of TCMTB into the top meter of the near shore area. The volume of water required to dilute this to the 96 h LC50 of 15 ppb is estimated at 11,900,000 L (or 2,600,000 gallons). This volume is equivalent to 1.2 ha (2.9 acres) one meter deep.

According to statistics maintained by Environment Canada's Atmospheric and Environment Service a storm of this intensity or greater would happen at least five times per year in this area.

5.3 The Concentrations of TCMTB in Fraser River Water When Effluent is Discharged Below the Surface of the River

Figure #7 shows the rainfall and TCMTB concentration at various depths in the Fraser River during a storm on January 9, 1990. During this event the surface of the river was up to 1.75 meters above the level of the outfall from the planed lumber storage yard.

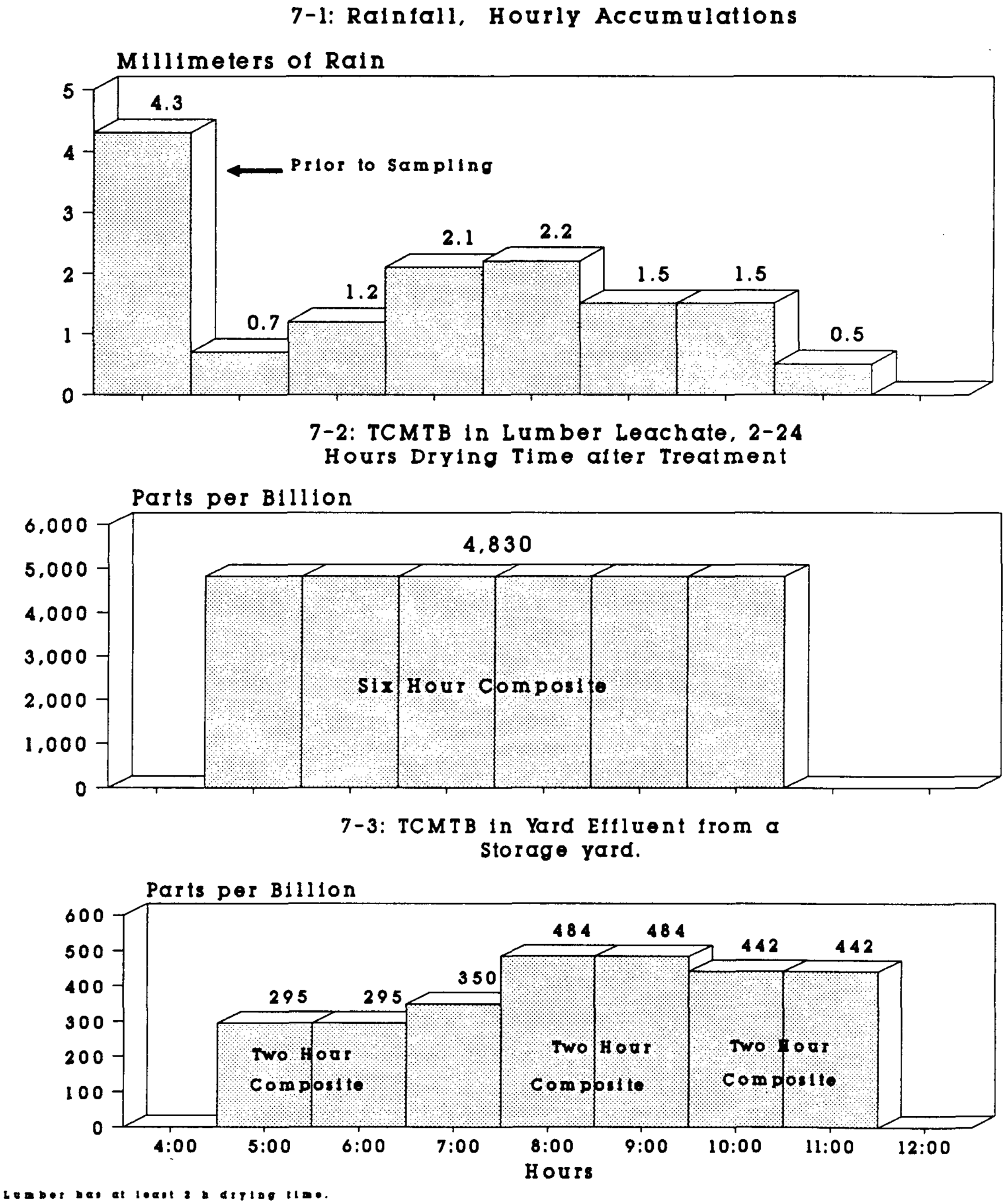
The rainfall profile in Figure 7-1 shows a continuous storm event which peaked at 2.2 mm/hr with a total accumulation of 14.0 mm. According to rainfall statistics for this area this type of event would occur at least 30 times per year.

Figure 7-2 shows the concentration of TCMTB in leachate dripping directly from the dressed lumber was 4830 ppb. This sample was collected as a single six hour composite in a 3.0 L tray. (Depending on the rain intensity and duration the capacity may have been exceeded.

Figure 7-3 shows the concentration of TCMTB in effluent discharging from the yard ranged from 295 ppb to 484 ppb with an average value of 392 ppb which is a 12 fold dilution of the lumber leachate. The TCMTB concentration profile showed the typical increase as rainfall intensity decreased.

Figure 7-4 shows the concentration of TCMTB in the river water five meters from the effluent discharge. Samples were collected simultaneously at three depths as follows: zero, one meter and two meters below the surface. Concentrations ranged from 19 ppb to 79 ppb with an average of 52 ppb at the surface; 6 to 15 ppb (average of 10 ppb) one meter below the surface and from nondetectable to 3.5 ppb two meters below the surface. (Note that 3.5 ppb is within the limits of detection but outside the 95% confidence interval). The

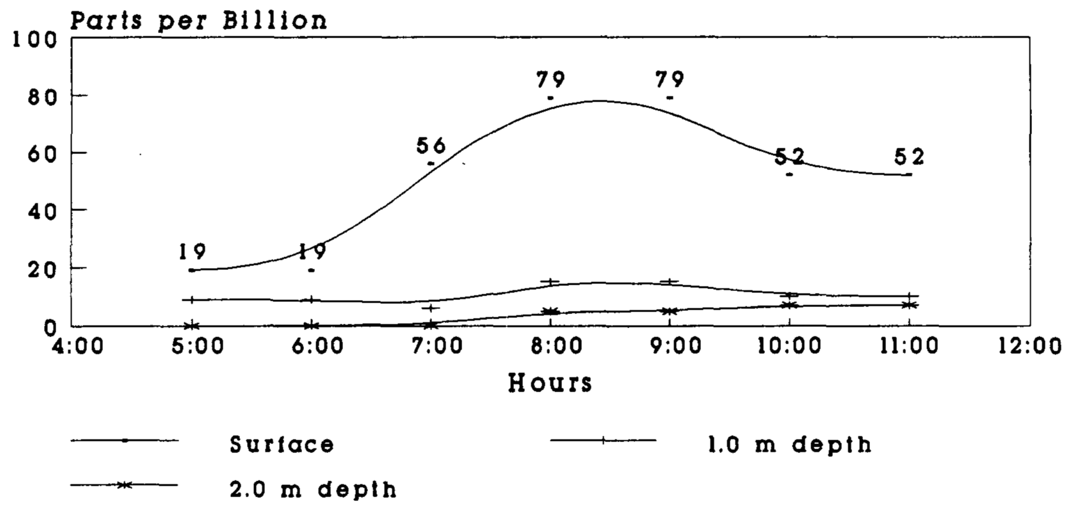
Figure 7: Rainfall vs Concentration of TCMTB in Runoff, Effluent and Fraser River Water



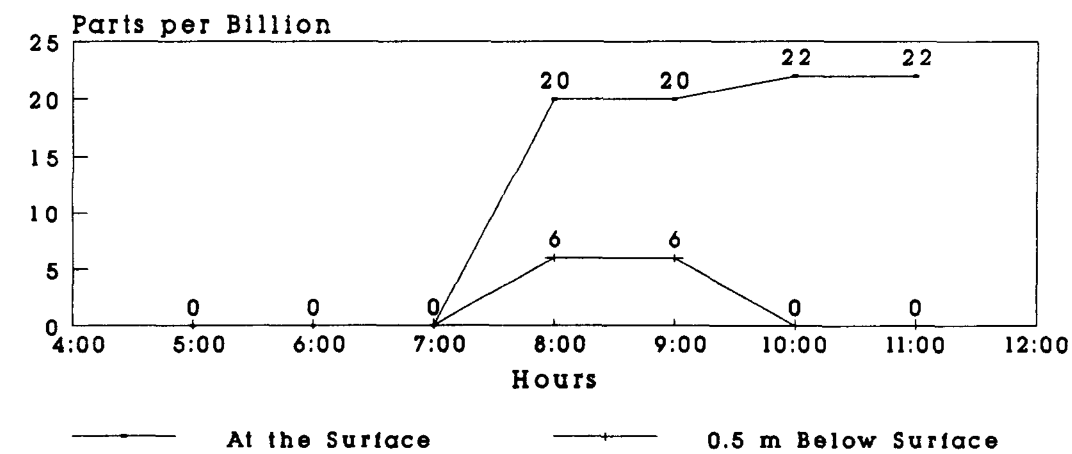
January 9,1990, Planed Lumber Storage Yard

Figure 7: Rainfall vs Concentration of TCMTB Continued

7-4: TCMTB in the Fraser River
5 meters from the Effluent Discharge



7-5: TCMTB in the Fraser River
10 meters from the Effluent Discharge

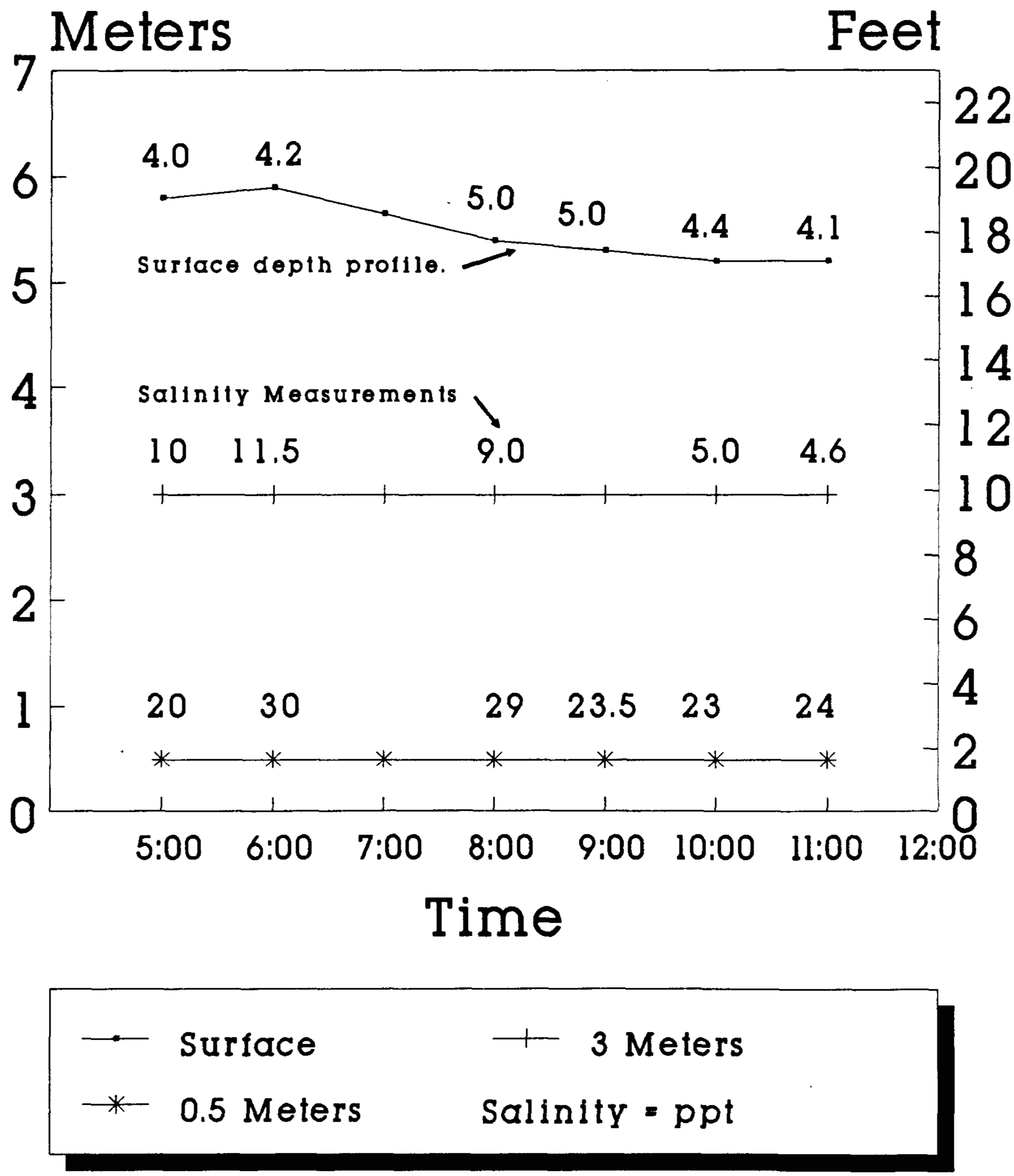


January 9, 1990, Planed Lumber Storage Yard concentration profiles at all three depths correlated directly with the concentrations in the effluent. The temperatures of the effluent and the Fraser River water averaged 6.43 C and 4.97 C respectively. The higher hydraulic head of the yard effluent created a continuous discharge to the river even when the tide was 1.7 meters above the level of the outfall. The warmer effluent temperatures caused the plume to reach the surface of the river within several meters of the discharge. The rising plume caused concentrations of TCMTB to be highest at the surface of the river.

Figure 7-5: The concentration of TCMTB in the surface river water ten meters from the effluent discharge was found to average 21 ppb at the surface and reach a maximum of 6 ppb one meter below the surface. The concentration profile at these locations appeared to lag behind those at the effluent discharge. This lag would be due to resistance of the movement of the plume in the main body of the river (i.e. The energy of the runoff water is dissipated as it slows and mixes with the slow moving river water). Figures 7.4 and 7.5 show that the effluent plume has risen to the surface and that the top meter of water in the Fraser River is subject to contamination for periods equivalent to or greater than the duration of each rainfall event. The longer duration was due to the dripping of the water saturated lumber packages after the storm has ended combined with infiltrating ground water which discharges from the pipe.

Figure 8: The salinity profile of the river was monitored at three depths during each sampling, surface (5.2 to 5.9 m from the bottom depending on the tide), 3.0 m from the bottom and at the bottom. The salinity at the bottom of the river was highest at high tide averaging 25 parts per thousand (ppt) and ranging from 20 to 30 ppt. At 3.0 meters from the bottom the salinity averaged 8.0 ppt ranging from 4.6 to 10 ppt. At the surface the salinity averaged 4.5 ppt ranging from 4.0 to 5.0 ppt over the seven hour monitoring period. The salinity tended to peak at the highest level of the tide and gradually decreased as fresh water from upstream began to displace the salt water wedge.

Figure 8 : Salinity at various depths in the Fraser River vs Time



January 9, 1990, 35 m from Discharge

6.0 DEGRADATION OF TCMTB

TCMTB can be degraded by sunlight, hydrolysis in water and micro-organisms in soil.(1,2,4) The purpose of this chapter is to compare field observations with information found in literature.

Gas measurements in the free space of storage barrels, tanks and in antisapstain mix rooms containing TCMTB indicate the presence of hydrogen cyanide HCN. Concentrations of 5 to 20 ppm were recorded in the free space of barrels.(4) HCN was not analysed in this study.

The products of hydrolysis, photolysis and methylation of TCMTB are illustrated in Figure 9. The combined actions of these various mechanisms may result in the formation of MMBT which may be bioaccumulative in the marine environment.(2) This mechanism produces MBT, thiocyanate ion and formaldehyde. The mechanism for photolysis is proposed in Figure 10.(1) Under laboratory conditions, potassium dihydrogen phosphate (KH_2PO_4) was dissolved in water and saturated aqueous TCMTB. The pH was adjusted to 6.00 with 0.1 M potassium hydroxide (KOH) and the solution was then stored in a clear borosilicate glass container and subjected to direct sunlight for two days. Analysis confirmed that less than 2% of the TCMTB remained. The experiment was repeated and analysis of the liquid after two hours of exposure to sunlight confirmed the presence of MBT. The yield of MBT was 50%. After seven hours of exposure the presence of BT in small quantities was confirmed.(1)

In Figure 10 the photolysis reaction proposed by Brownlee is compared with the concentration of TCMTB, MBT, BT and MMBT found in a leachate sample from treated wood. The field measurements occurred on November 3, 1989 in a storage area which contained lumber that had been stored for such a long period that mould was beginning to grow at the ends of the boards.(Table #5, Plates #3,#4) The working solution at the study mill had an average concentration of 4,000,000 ppb TCMTB and was applied at an average rate of

Figure 9: Proposed Life Cycle of TCMTB

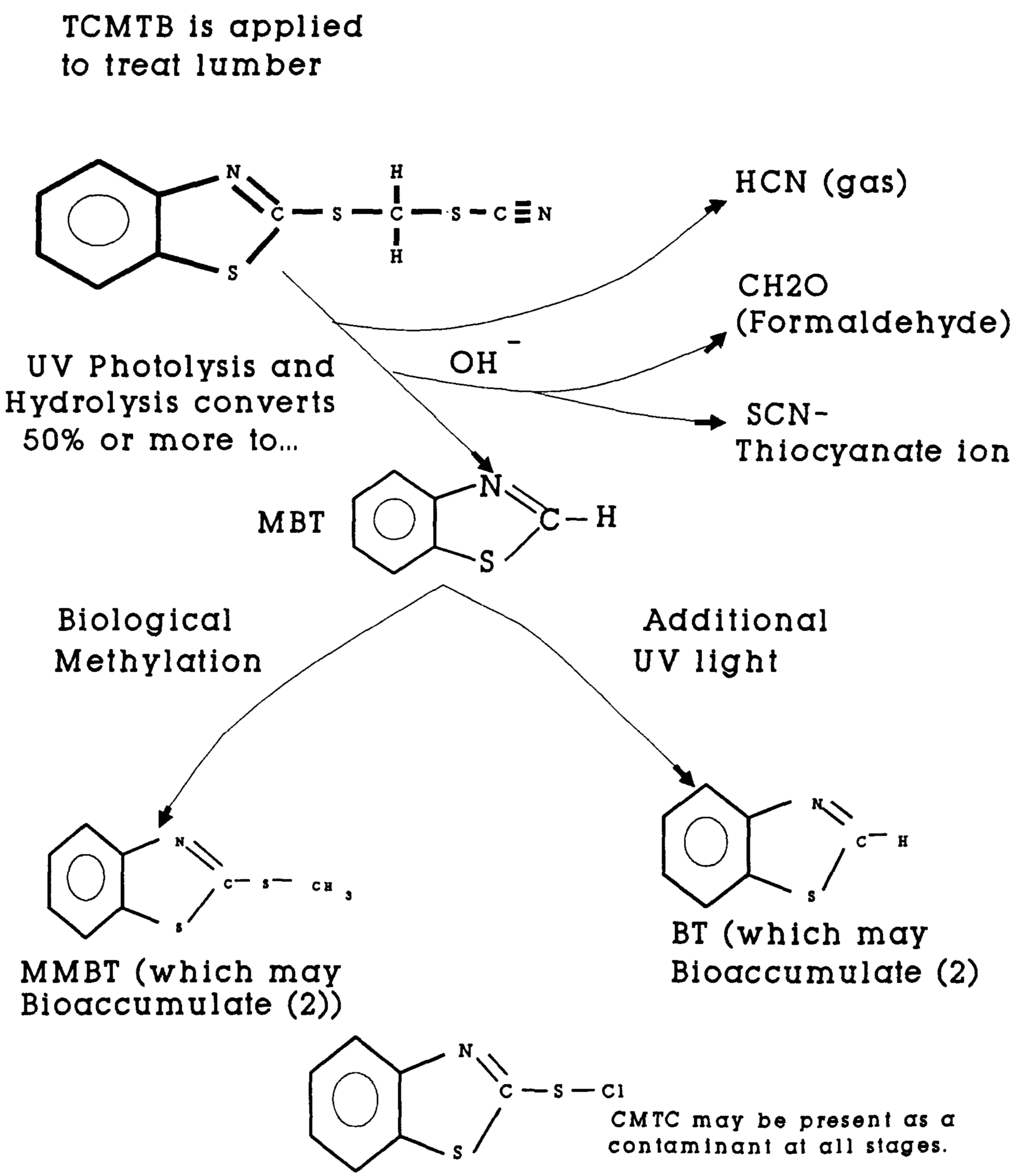
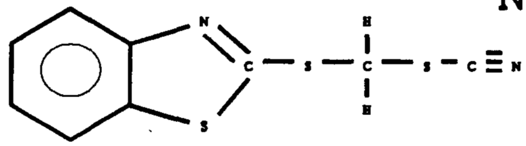


Figure #10: Degradation of TCMTB via Photolysis Laboratory Conditions vs Field Observations

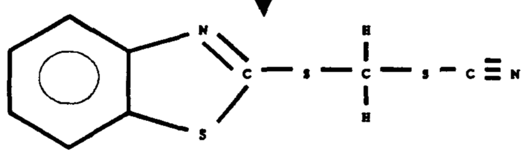
Laboratory Conditions (1)		Field Conditions Nov. 3, 1989



TCMTB = 8,300 ppb
+ KH_2PO_4
Add KOH to pH=6.0
+ UV Light for two days

Natural Wood Acidity
UV light and rainfall fluctuate over several weeks

166 ppb TCMTB

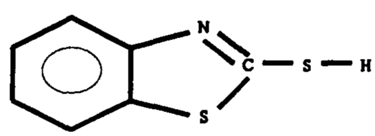


229 ppb

+

MBT

50 % Yield (4,150 ppb)

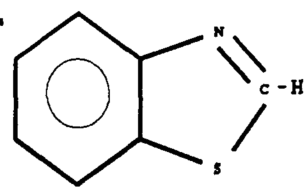


4,740 ppb

+

BT

Trace (Value not given)

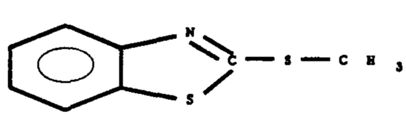


Less than 20 ppb

+

MMBT

Not Analysed For



33 ppb

61.3 ug/cm^2. The pH of the wood is slightly acidic (9) and the repeated cycling of rain and sunshine would have provided similar conditions of moisture, pH and UV light as was provided in the Brownlea experiment over an extended period of time. (The pH of the rain was not measured).

The concentration of TCMTB in leachate dripping from treated wood averaged 4715 +/- 2572 ppb (see section 4.4), however on Nov. 3, 1989 it was only 229 ppb. The concentration of MBT in this sample was 4740 ppb which would indicate an approximate conversion of 90% from TCMTB. BT was below detection limit and MMBT was 33 ppb.

The source of the MMBT was not determined. TCMTB and its metabolites were monitored in the working solutions in the mixroom on five occasions from November 3, 1989 to January 9, 1990. MMBT was always detected in the working solution with an average concentration of 9352 +/- 5155 ppb. The concentrate solution was not tested for metabolites so it is not known if it was the source of the MMBT in those samples.

On November 3, 1989, the freshly treated lumber was leaching at a very high rate of 89,800 ppb TCMTB but did not contain any MBT or BT and only 130 ppb of MMBT (Table 2). If this was the only source of MMBT the yard concentrations would have been well below those tested in Table 5 therefore degradation or bacterial action on the surface of the treated lumber may be the source. All other samples collected which received leachate or yard runoff from the old treated lumber storage areas were high in MBT.

Table 5 shows the concentrations of TCMTB, MBT, BT and MMBT in the rough cut lumber storage yard runoff during the month of November 1989. During this time period the inventory of the old stained (mouldy) lumber was used up and the concentration of MBT steadily decreased from 1220 ppb to nondetectable levels by December 2, 1989.

7.0 PREDICTING THE CONCENTRATION OF TCMTB IN RUNOFF FROM A TREATED LUMBER STORAGE YARD

There are two main factors to consider in the calculation of the concentration of TCMTB in the runoff from a treated lumber storage yard: the application rate of the chemical and the quantity of treated lumber on the storage yard. The application rate of TCMTB vs the concentration in the leachate from lumber subjected to rainfall was compared for two different data sets at two separate treatment facilities. At one site the lumber was treated via dipping which achieved an application rate of 25 +/- 13 ug/cm2 whereas at the other site an average application rate of 61 +/- 20 ug/cm2 was achieved by high pressure spraying. Both sets of lumber were allowed to dry 24 hours before exposure to natural rainfall at different sites over different rainfall events. Samples of leachate were collected after each rainfall event and analysed for TCMTB. The results are plotted in Figure 11.

It was assumed that TCMTB will not leach from the lumber if none is applied for antisapstain purposes. There is no other known source for TCMTB in the lumber production process and therefore the origin will be part of the data set. The plotted points suggest a linear relationship of the form:

$Y_L = mX + b$ where;

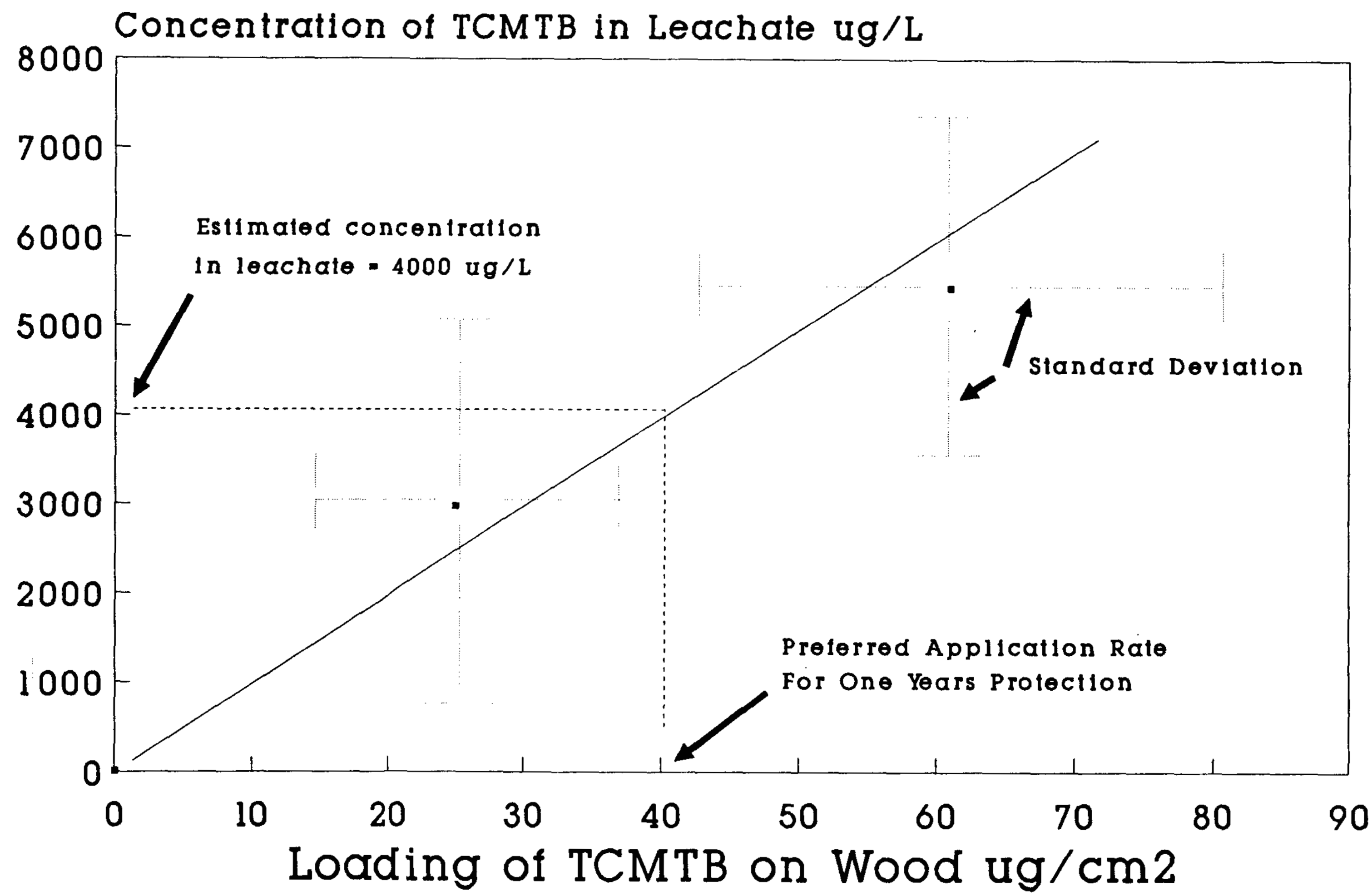
Y_L = the concentration of TCMTB in the leachate in ug/L (parts per billion)
X = the application rate of TCMTB in ug/cm^2
m = the slope of the line defined as $\frac{Y2 - Y1}{X2 - X1}$
b = the Y intercept (which in this case is the origin)

Using the least squares method to determine the line of best fit the slope was found to be 95.5 which yields the formulae:

$$Y_L = 95.5X$$

Using data from Tables 2,3,4 and 5 and Ref #6 it was determined that an average dilution of 12.88 occurred on the mill yard from the time the leachate

Figure 11: Loading of TCMTB on Wood vs Concentration of TCMTB in Leachate



struck the pavement to the point of discharge. A review of all the data collected by Environment Canada over 16 sampling sequences at operating antisapstain facilities determined an industry dilution ratio 15 +/- 9 times.(10) The $\pm$ 9 refers to one standard deviation in the data caused by variation in conditions at operating sawmills such as different lumber sizes, storage area loading rates, species, and different growths. Each of the 16 rain events was unique in accumulation, intensity and duration. This is therefore an industry wide equation. Applying this to the formula above the concentration in the effluent from a mill yard can be estimated using the formula;

$$YR = \frac{YL}{D} = \frac{95.5X}{(15 \pm 9)}$$

For a mill applying the recommended 40 ug/cm^2 of TCMTB the average yard runoff would be:

$$YR = \frac{YL}{D} = \frac{95.5(40)}{(15 \pm 9)} = 255 \text{ ug/L}$$

Under normal operating conditions the average yard runoff from a treated (planed) lumber storage yard is estimated to be 255 ug/L. The range in concentrations at any given time based on one standard deviation of the dilution rate is 160 to 640 ug/L.

If the concentration of TCMTB in yard runoff which drains into the nearest catchment basin is higher than the range of 160 to 636 ug/L, it would indicate a misuse of the chemical. The most common misuse is insufficient drying time and Figures 6-2 and 6-3 are typical examples of such a misuse.

This equation indicates that a lumber treatment facility designed to comply with the current code of practice, will exceed the British Columbia Antisapstain Chemical Waste Control regulation limit of 15 ug/L. In order to achieve this limit, improvements such as wrapping, roofing or reformulation are required.

BIBLIOGRAPHY

1) Brownlee, B.G., "Environmental Dynamics of Benzothiazole Based Pesticides", Rivers Research Branch, National Water Research Institute, Burlington, Ontario, April 1989.

2) Spies, Robert B., Brian D. Andresen, David W. Rice Jr., "Benzothiazoles in Estuarine Sediments as Indicators of Street Runoff", Nature, Vol. 327, No. 6124, pp.697-699, 25 June 1987.

3) Byrne, A., D.R.d Minchin, "Leaching of TCMTB from Packaged Lumber", Forintek Canada Corp., Western Region, Report Prepared for Buckman Laboratories of Canada, Dorval, Quebec, August 1989.

4) Agriculture Canada, Health and Welfare Canada, Environment Canada, Department of Fisheries and Oceans, Canadian Forestry Services, "Discussion Document on Anti-Sapstain Chemicals", October 1989.

5) Buckmans Chemicals, "Guidelines to the Safe Use of TCMTB".

6) Personal Communication, Dr. George Kruzynski, Department of Fisheries and Oceans, West Vancouver Laboratory.

7) Birckbeck, A.E., Reil, D.D., "Assessment of Penetration of TCMTB into Concrete and Asphalt Areas at the Frser Surrey Dock", Division of Applied Biology, B.C. Research, 3650 Westbrook Mall, Vancouver, B.C. V6S 2L2.

8) B.C. Ministry of the Environment, "Antisapstain Chemical Waste Control Regulation", Waste Management Act, 1989.

9) Chorophenate, Recommendations for Design and Operation, Environment Canada, B.C. Ministry of the Environment, 1984.

10) Krahn, P.K., "Standard Leaching Test for Antisapstain Chemicals", Environment Canada, Environmental Protection, Pacific and Yukon Region, Regional Program Report 90-10, 1990.

11) Krahn, P.K., "An Estimate of the Concentration of 2(thiocyanomethylthio) benzothiozole (TCMTB) in Storm Water Runoff from Treated Lumber Storage Yards Which Comply to the Code of Good Practice". Regional Data Report, August 6, 1988.

12) Forintek Canada Corp., "Simulated Rainwater Leaching of Chlorinated Phenols from Dip Treated Lumber". January 1989.

13) Forintek Canada Corp., "Leaching of Didecyldemethyl Ammonium Chloride and Borates from F2 Spray Treated Lumber". October 1989.

14) MacMillan Bloedel Research, "Mill Antistain Treatment Using TCMTB".

ACKNOWLEDGEMENTS

The author wishes to express his appreciation to the following people for their excellent contributions to this study.

Richard Strub	Environment Canada, Organic Chemist
Amy Palmer	Environment Canada, Technical Analyst
Richard Glue	Environment Canada, Project Engineer
Emmanual Mendoza	Mendoza Consulting, field work
John Alexis	Alexis Consulting, field work
Lydia Latourneau	Envirochem Services Ltd, field work, mill liaison
Heather Hindle	Environment Canada, word processing
Ken Wile	Environment Canada, editorial and policy review

APPENDIX I

ANALYTICAL METHOD FOR

2-(THIOCYANOMETHYLTHIO)BENZOTHIAZOLE (TCMTB)

Environment Canada
Conservation and Protection

Laboratories
Pacific & Yukon Region

2-(Thiocyanomethylthio)benzothiazole — V1.2 March, 1990 — SAPLC

High Performance Liquid Chromatography

SCOPE AND APPLICATION

This method is applicable to the determination of 2-(Thiocyanomethylthio)benzothiazole (TCMTB) and the metabolites Benzothiazole (BT), 2-Mercaptobenzothiazole (2MBT) and 2-Methylmercaptobenzothiazole (MMBT) in effluents and sediments. The TCMTB, BT, 2MBT, and MMBT detection limits in effluents are 1, 10, 25, and 5 ug/l, respectively. Sediment detection limits are currently under evaluation.

SAMPLE HANDLING AND PRESERVATION

Note: All glassware, aluminum foil and sampling equipment that comes in contact with the sample must be acetone rinsed and heat-treated at 325 C for a minimum of 12 hr.

Samples should be collected in an all glass system and stored at 4 C or just above the freezing point. If Teflon lined caps are not available for the sampling containers then heat-treated aluminum foil must be used as a liner. At least 1 litre of sample should be collected.

SUMMARY OF METHOD

The sample is first extracted with methylene chloride then the extract is filtered, concentrated and the residue dissolved in acetonitrile. The sample is then analysed for TCMTB and its metabolites MBT, MMBT and BT using High Performance Liquid Chromatography (HPLC).

METHOD PERFORMANCE

1. Five sample replicates yielded a 7.5 Average % Standard Deviation.
2. A DI water solution spiked with TCMTB at the 20 ng/l gave the following average recovery with a Standard Deviation of 7.40 over 5 replicates.

Compound	Actual value (ng)	Found value (ng)	% Recovery
TCMTB	2.8	2.7	95.9%

3. The linear coefficient of correlation for each TCMTB standard was better than 0.996 over a 1 - 43 ng Injection Solution range (5 ul injection).

contd. ...

INTERFERENCES

1. Interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing hardware that lead to discrete artifacts and/ or elevated baselines in HPLC chromatograms. All materials must be routinely demonstrated to be free from interferences under the conditions of analysis.
2. Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature and diversity of the industrial complex or municipality being sampled.
3. Store standards at 4 C and protect from light. Stock standards should be checked frequently for signs of degradation.
4. Compounds co-eluting with TCMTB and absorbing UV at 280 nm are potential sources of interference.

SAFETY

TCMTB should be treated as a potential health hazard. Exposure should be kept at the lowest level possible. Use well vented fume hoods and wear protective clothes and gloves at all times when using these chemicals.

APPARATUS

All glassware must be washed with heavy-duty soap, rinsed with distilled water, rinsed with acetone and then heat-treated at 325 C for at least 12 hours prior to use. The glassware must be stored in an area protected from chemical contamination.

1. Graduated cylinders, 500-ml.
2. Boiling flasks, 500-ml.
3. Separatory funnels, 1-litre.
4. Vials, 7-ml. with foil cap liners.
5. Volumetric flasks, 2-ml, 10-ml.
6. Rotary evaporator.
7. Centrifuge.
8. Glass columns, 26 cm x 2.0 cm, with sintered glass frits and teflon stopcocks.
9. Visiprep Solid Phase Extraction Vacuum Manifold, Supelco #5-7030.
10. Supelclean Solid Phase Filtration Tubes, Supelco #5-7026.
11. Buchner funnel, 5.0 cm.
12. Vacuum adapter, 24/40.
13. GF/C filter paper, 4.5 cm.
14. Burrel wrist-action shaker.

contd. ...

APPARATUS (ctd.)

15. High performance liquid chromatograph, Hewlett-Packard 1090 equipped with diode-array detector and temperature controlled autosampler. HPLC column, Hewlett Packard, ODS-HYPERSIL(C18), 5um, 200mm x 2.1 mm i.d.(microbore).
16. Autosampler vials and caps, 2-ml.

REAGENTS

1. Methanol, Burdick & Jackson HPLC grade, filtered through Nylon 66 membrane filter (0.2 u x 47 mm diameter).
2. Methylene chloride, Burdick & Jackson HPLC grade.
3. Acetonitrile, Burdick & Jackson HPLC grade, filtered through Nylon 66 membrane filter (0.2 u x 47 mm diameter).
4. Deionized water, filtered through Nylon 66 membrane filter (0.2 u x 47 mm diameter).
5. TCMTB (99.3%), Buckman Laboratories, P.O. Box 8305, Memphis, Tennessee, 38108.

PROCEDURE - GENERAL

1. Blanks are required for each new batch of reagents.
2. Duplicates and spiked samples must be run on a routine basis.
3. Standards should be injected frequently as a check on the stability of operating conditions.

PROCEDURE - EXTRACTION OF EFFLUENTS

1. Add 500 ml of the shaken sample to a 1-litre separatory funnel.
2. Extract TCMTB and metabolites by adding 75 ml methylene chloride and vigorously shaking the separatory funnel for 2 minutes.
3. Extract twice more with 75 ml of methylene chloride, draining the solvent fraction through a funnel plugged with glass wool and 5 g Na_2SO_4 into a 500-ml round bottom flask after each extraction.
4. Rotary evaporate the solvent to dryness then quantitatively transfer the residue to a 2-ml volumetric flask with acetonitrile by filtering through a Supelco filter tube and filtration apparatus.
5. The sample is now ready for analysis.

PROCEDURE - EXTRACTION OF SEDIMENTS

1. Air dry approximately 50 g of the sample overnight. Weigh 25 g of the air-dried sample into a 100-ml heavy-walled shaker tube, add 50 ml of methylene chloride and shake the sample on a wrist-action shaker for 2 hours.

contd. ...

PROCEDURE - EXTRACTION OF SEDIMENTS (ctd.)

2. Vacuum filter the methylene chloride extract through a Buchner funnel with a vacuum adapter using Whatman #1 filter paper and collecting in a 500-ml round bottom flask. Rinse the shaker tube twice, each with 25 ml of methylene chloride and filter as above.
3. Add approximately 5 g of heat treated sodium sulfate to the flask, then cover and let the extract stand for 30 min.
4. Transfer the extract to a clean 500-ml round-bottom flask and rotary evaporate to dryness.
5. Quantitatively transfer the residue to a 2-ml volumetric flask with acetonitrile by filtering through a Supelco filter tube and filtration apparatus.
6. The sample is now ready for analysis.

PROCEDURE - HPLC START-UP

NOTE: All control of the HPLC should be from the HP 300 computer.

1. Ensure the HP 1090 mainframe is turned on then turn on the computer.
2. Check the solvent and distilled water reservoirs. Use freshly deionized water daily. Turn on Helium for solvent degassing.
3. Turn on the circulating bath for cooling of the autosampler.
4. Empty the waste solvent reservoir daily.
5. Check for sufficient printer paper.
6. Once computer has been booted it will show that you are at TOP LEVEL. Press the (F2) soft key, <DATA ACQUIS>. To move to different areas on the screen use the <TAB> key.

PROCEDURE - SET UP OF HPLC SEPARATION PARAMETERS

1. Once at <DATA ACQUIS> level press (F6) soft key, <EDIT PARAMS>. To get the TCMTB conditions press (F1), <LOAD PARAMS>, press <?><RETURN>, and (F1), <SELECT ITEM> TCMTB.A. This loads all the liquid chromatograph and diode array detector conditions required to separate TCMTB and its metabolites BT, 2MBT, and MMBT. Press (F8), <EXIT>.
2. Press (F7), <MORE KEYS>, press (F3), <PUMP ON>, press (F7), <MORE KEYS>, Press (F3), <MONITOR>. The wavelength screen will have spurious peaks and must be balanced by pressing (F6), <BALANCE> key. A flat baseline should then be obtained. DO NOT PROCEED UNTIL THIS IS CORRECT. Then press (F8), <EXIT> and then (F8), <QUIT>.
3. Once at TOP LEVEL again, press the (F5), <SEQUENCE> soft key. The cursor key will be at the sequence table line, press <?><RETURN> and (F1), <SELECT ITEM> T1.R. Tab down to Sample Table file name, press <?><RETURN> and (F1), <SELECT ITEM> T1.S. Tab down to Data file name and set your

contd. ...

3.(ctd.)
Data file as follows: If running multiple samples and injections the file name is replaced with _____00A. The first 5 characters you can select as the descriptor. The last 3 characters (00A) are used by the Chem Station as a counter for the vial and injection numbers.

ex. _____00A.D - Vial #0 (00) injection number 1(A).
_____02A.D _ Vial #2 (02) injection number 1(A).

Use an "A" for the fifth character of the Sequence run for automated runs. For routine samples the last four characters of the Laboratory Sample Number should be used (ex. 0833AxxA.D). The counter sequence will identify the sub-sample. When running an unattended analysis on the HPLC start at Sequence Line A then tab down to operator name and fill in as appropriate.

4. Press (F1) soft key, <EDIT SEQ FILE>, then <?><RETURN> and (F1), <SELECT ITEM> TCMTB.M. Set the Range with how many samples are to be run and where they are situated in the auto-sampler (i.e. Range 00:30 Rep 1 represents sample vial 1 to sample vial 30 with one injection). If any existing sequence files are present they can deleted by using the space bar and erasing the existing files. Press (F8), <EXIT> from Seq file.
5. Press (F2) soft key, <EDIT SAMPL TBL>. This table identifies every sample and how it is going to be analyzed (i.e. either a sample or calibration standard). Start by indexing the sample number, sample identification and then pressing (F5), <MORE INFO> to give the sample more identification. In this section you put in any dilution factor incorporated in the sample, is it a calibration sample or not. If it is a calibration standard, at what level is it at with your existing calibration curve. If it is a recalibration standard you can either replace the level or average the level. Do this for every sample wanted to be analyzed. As in the Sequence file any existing samples in the table can be deleted by "Space Barring" through the Sample table and erasing existing samples.
6. Press (F8), <EXIT>, then press (F6), <CHECK METHODS>. If all is correctly set up you can press (F4), <START SEQUENCE> and unattended run can proceed.
7. Once START SEQUENCE has commenced press (F7), <HOLD METHOD> and check that the DATA ACQUIS FILE and all the conditions are correct. Check that the right vial is being injected. If condition are not met press (F8), <STOP METHOD>, wait for the first run to finish and recheck all parameters.
8. Note that you can check the integrity of your standard curve by re-running standards and "addressing" them in the sample table (see 5.).
9. Press (F8), <RESUME> once everything is correct and run the samples.
10. The REPORT GENERATOR will provide all the quantitation results and report if the sample was a recalibration standard.

contd. ...

PROCEDURE - HPLC ANALYSIS

1. Liquid Chromatograph Conditions:

FLOW:	0.50 ml/min	STOP TIME:	10.0 min
SOLVENT A:	50% H20	POST TIME:	3.00 min
SOLVENT C1:	50% ACETONITRILE	INJ. VOL.:	10.0 ul
OVEN TEMP:	40.0 C		

GRADIENT CHANGE: TIME 0.0 min = 50% Acetonitrile.
TIME 10.0 min = 100% Acetonitrile.

DIODE ARRAY DETECTOR CONDITIONS:

SIGNALS: A

SAMPLE:	Wavelength = 281nm	REFERENCE:	Wavelength = 550nm
	Bandwidth = 4nm		Bandwidth = 100nm

Store the spectrum under peak controlled conditions with a threshold of 0.4 mAU, and a peakwidth of 0.100 min.

2. Inject exactly 10.0 ul of the TCMTB Standard Mixes T1 - T4 in the appropriate concentrations and injected on the HPLC for confirmation of individual peak identification by retention time and full diode array library match.

TCMTB and METABOLITES	CONCENTRATION (ng/ul)			
	T1	T2	T3	T4
TCMTB	132.0	66.0	33.0	6.6
2-Mercaptobenzothiazole	118.0	59.0	29.5	5.9
Benzothiazole	115.0	57.5	28.8	5.8
MMBT	154.0	77.0	38.5	7.7

3. Inject exactly 10.0 ul of each sample and collect data.

contd. ...

CALCULATIONS AND DATA PROCESSING

[SAMPLE CONC.] = {[INJECTION SOLUTION CONC.] X [A] X [B] X [C]}

INJECTION SOLUTION CONC. = from Quantitation Report, in ng/ul
A = Final Volume of Injection Solution, in ml.
B = Dilution Factors (if any).
C = for Effluent Samples = [1000]/[Volume Sample extracted - in ml]
= for Sediment Samples = [1]/[Dry Wt. Sample extracted - in g]

Reporting Units: for Effluent Samples = ug/l
: for Sediment Samples = ug/g

The concentrations of TCMTB, BT, 2MBT, and MMBT are entered in the laboratory database using "RENT" software and entering "SAPLC" for the Package (Billing) Code.

REFERENCES

[1] Hewlett Packard HP1090 Liquid Chromatograph Fact File Handbook, First Edition, November, 1987.
[2] Stability of TCMTB Anti-Sapstain Solutions, Prepared by B.C. Research, April, 1985.
[3] 2-(Thiocyanomethylthio)benzothiazole in Air, Worker´s Compensation Board of British Columbia, 20 Feb., 1985.
[4] Analytical Method of TCMTB (communication), Buckman Laboratories International, Inc., July 6, 1988.
[5] Environmental Dynamics of Benzothiazole-Based Pesticides, B.G. Brownlee, Rivers Research Branch, National Water Research Institute, April, 1989.
[6] Benzthiazoles in estuarine sediments as indicators of street runoff, Robert B. Spies, Brian D. Andresen & David W. Rice Jr., Nature, June 25/87.

REVISION HISTORY

Version 1.2	MAR/90	Change to microbore HPLC column resolves all metabolites analyzed for. ECD-GC and GC/MS procedures no longer required.
1.1	DEC/89	Documentation upgrade, addition of MMBT by ECD-GC & GC/MS.
1.0	NOV/88	New method.

contd. ...

PROGRAM USED BY HPLC COMPUTER SYSTEM (H/P 300 CHEMSTATION) TO IDENTIFY PEAKS FOR PURITY AND LIBRARY MATCH BASED ON SPECTRAL LIBRARY FIT AND RETENTION TIME

```
!-----------------------------------------------------------------!
!   Macro for automated spectral library search                   !
!                                                                 !
!     Signal with peaks in z-register.                            !
!                                                                 !
!   AUTOLIB.G Version 4.01                                        !
!      First release.                                             !
!   AUTOLIB.G Version 4.06                                        !
!     If sequence running then no query for input and default     !
!       settings (library is taken from method data file param)   !
!-----------------------------------------------------------------!

NAME AUTOLIB
  seqinf               ! sequence info
  CHR ,81
  EX X,Z
  gets header,z
  If Nobjects=0 Then
    Convert A$,"No signals in Z-register, please select first"
    lib_ABORT               ! return to data editor macros
    RETURN
  EndIf
  Strpos "AUTOLIB 4.06 Initialized",AutolibIn$
  if len1=len2 then
    if value=1 then
    else
       INITAULIB     ! first time so initialize variables
  endif
else
       INITAULIB     ! first time so initialize variables
  format merged
  clear
  cl x
  cl y
  cl t

  if seq_run=0 then   ! no method or sequence so ask
    Convert A$,"N"
    Getvar A$,"Change settings (No)"
    strpos "Y",A$
    if value>0 then
```

contd. ...

```
        SETVAR          ! change settings
      endif
      DADlib FILE
    else                ! sequence/method running
      DADlib FILE,,NOQ  ! open default library
    endif
    ex x,z
    ent
    mul 1
    DRAW sigtowin,y
    filestart=xlow
    fileend=xhigh
    ref_time=filestart
    spec filend
    lastspec=scan_num   ! get last spectrum available
    gets header,z
!   dychar=charsize/10*(yhigh-ylow)/40
    dychar=charsize/10*(yhigh-ylow)/(40-fieldsize-7)  !???????????
    ysc=yhigh+5*dychar
    if numpeaks=0 then
      INTEGRATE Z
      numpeaks=npeaks
    endif
    PeaksFound=NumPeaks
    If PeaksFound=0 Then
    Convert A$,"No peaks found; please change int params first"
      lib_ABORT
      RETURN
    EndIf

    if 1=2 then
      size=0
      xl=0.5
      while size<20
        annot sigtowin,0,size,0,xl,0.3,"C&P Library search"
        size=size+1
        xl=xl-0.01125
        annot sigtowin,0,size,3,xl,0.3,"C&P Library search"
      endwhile
    endif

    if doprint=1 then
       PRHEAD
    endif
```

contd. ...

```
CurPeak=0
dr sigtowin,y
format merged
while CurPeak<Peaksfound
  CurPeak=CurPeak+1
  MESS "Peak spectra search....                          ",,0,-5
  PEAKS CurPeak,,none,Z,1,st_wave:en_wave
  Crest=Ret_Time
  rol -1
  spec ret_time,st_wave:en_wave
  top_time=ret_time
  if (ret_time-start_time)*(end_time-ret_time)>=0 then

    Convert A$,"Peak#",CurPeak:4:0,"  time:",RET_TIME:7:3
    mes A$,,0,-6

   !--  get the spectr/a/um  --!
    if dopeakpur=1 then
      PEAK_SPEC
    else
      if dosubref=1 then
        if ref_spec<0 then
           PEAK_SPEC
        endif
      endif
    endif

    !-- subtract reference spectrum  --!
    if dosubref>0 then
       SUBREF
    endif

    !-- remove empty objects  --!
    gets header,x
    objs=nobjects
    i=1
    while i<=objs
      gets header,x,i
      if ylow=0 then
        if yhigh=0 then
          extr i
          rol -1
          i=i-1
```

contd. ...

```
        objs=objs-1
      endif
    endif
    i=i+1
  endwhile

  gets header,x
  if nobjects>0 then

    if nobjects=2 then
      if dopeakpur=0 then
        extr
        add
        mul 0.5
     endif
    endif
     SMDERNORM
    gets header,x
    if nobjects>1 then
      dr purtowin,x
      compare ab_thres,0,x,1,x,nobjects
      Convert A$,"Purity:",match:6:1
      ann purtowin,,,,,,A$,xaxis
      dr purtowin,x
      peak_pur=match
      if match>purthres then
        if dosubref>0 then
          if ref_spec<0 then
            extr
            add
            mul 0.5
          else
            ro -1
            spec top_time,st_wave:en_wave
         SUBREF
          endif
        else
          ro -1
          spec top_time,st_wave:en_wave
        endif
         SMDERNORM
      else
```

contd. ...

```
    DADLib SEARCH,SPECTRUM,ab_thres,cor_sh,leftwin:rightwin,x,nobjects
  endif
else
  peak_pur=-1000
endif
DADLib SEARCH,SPECTRUM,ab_thres,cor_sh,leftwin:rightwin,x,1
if doprint=1 then
  write 701,CurPeak:2:0,crest:9:2
  if dopeakpur=1 then
     if peak_pur>-0.1 then
       writereal 701,peak_pur:6:0
    else
      write 701,"   ???"
    endif
 else
    write 701,"   ---"
  endif

  upslope=0
  downslope=0
  DADlib RES,1,x,1
  if entry>0 then
    n_object=1
     gets header,x
    if nobjects>1 then
      upslope=1
    endif
     PRSEAR
    upslope=0
  else
    writeln 701,match:12:0
    endif
    gets header,x
    if nobjects>1 then
      n_object=nobjects
    downslope=1
     PRSEAR
    downslope=0
  endif

  mathfunc trunc,CurPeak/5,x
  if CurPeak-(x*5)=0 then
    writeln 701,""
  endif
endif
```

contd. ...

```
gets header
if nobjects=1 then
  convert A$,""
  DADlib RES,1,x,1
  high_match=match
else
  if peak_pur>purthres then
    convert A$,""
  else
    convert A$,"*"
  endif
  high_match=0
  DADlib RES,1,x,1
  If entry>0 then
    high_match=match
  endif
  DADlib RES,1,x,nobjects
  If entry>0 then
    if Match>high_match then
            high_match=match
  else
  DADlib RES,1,x,1
  high_match=match
    endif
  endif
endif
If entry>0 then
  if high_Match>=searthres then
    DADlib READ,entry,st_wave:en_wave
    convert A$,A$,Comp_name$
    norm 100
    if cor_sh=0 then
    else
      gets header
      trans xlow:xhigh,(xlow+cor_sh):(xhigh+cor_sh)
    endif
    merge

    dr spetowin
    SCREENPRINT 640,500
    mess "Entry#:",entry,0,-5
    mess "high_Match:",Match,20,-5
  else
```

contd. ...

```
            convert A$,A$,"?"
          endif
        else
          convert A$,A$,"?"
        endif
        if adjanot=1 then
          gets header,y
          gets point,start_time:end_time,z
          ymax=y+0.5*dychar
        endif
        if ymax+((fieldsize+5)*dychar)>ysc then
          ysc = ymax+((fieldsize+5)*dychar)
        endif
        AN sigtowin,anotrot,charsize,3,crest,ymax,A$[1:fieldsize],DATA,1
      endif
    endif
  endwhile
  Convert A$,"Library : ",lib_name$
  AN sigtowin,,,6,0.15,0.90,A$
  AN sigtowin,,,6,0.15,0.94,"Results of spectral library search"
  Convert A$,"Match > ",searthres:3:0
  an sigtowin,0,10,3,0.02,0.01,A$
  clear
  gets header,y
  dr 3,y,,ylow:ysc*0.8
  if doprint=1 then
    write   701,"---------------------------------"
    writeln 701,"---------------------------------"
    writeln 701,""
    makeprint
  endif
  CL
  CHR ,81
  EX X,Z
  EV TCMTB.E
  START SET
  INT Z
  DRAW 3,Z
  GETS Z
  DRAW 3,Z,,0:YHIGH*1.2
  PAGES 1
  DRAW PRINTER:,Z,,0:YHIGH*1.2
  QUIT
  RETURN
```

contd. ...

```
NAME MAKEPRINT
  win 4,0:1,0:1
  clear
  gets header,y
  dr 4,y,,ylow:ysc
  screenprint 640,500

  Clear
  dr 3,y,,ylow:ysc
  return
NAME MAKEPLOT
  win 4,0:1,0:1
  clear
  gets header,y
  dr 4,y,,ylow:ysc
  redraw 705

  Clear
  dr 3,y,,ylow:ysc
  return
!-----------------------------------!
!                                   !
!-----------------------------------!
NAME CHECKWAVE
  scan 1
  rol -1
  gets header,t,1
  if en_wave<=st_wave then
    st_wave=xlow
    en_wave=xhigh
  else
    if st_wave<xlow then
      st_wave=xlow
    endif
    if en_wave> >xhigh then
      en_wave=xhigh
    endif
    if en_wave<=st_wave then
      st_wave=xlow
      en_wave=xhigh
    endif
  endif
  RETURN
```

contd. ...

```
!----------------------------------------!
! change settings                        !
!----------------------------------------!

NAME SETVAR
  if leftwin>-0.01 then
    Convert A$,"Yes"
  else
    Convert A$,"No"
  endif
  Convert B$,"Library time search window (",A$,") >"
  getvar A$,B$
  strpos "Y",A$
  if value>0 then
    if leftwin<0 then
      leftwin=5
    endif
    Convert A$,"Search window (% of ret. time) (",LeftWin:0:1,") >"
    Getvar LeftWin,A$
    rightwin=leftwin
  else
    leftwin=-1
    rightwin=-1
  endif
  if dosubref=1 then
    Convert A$,"Yes"
  else
    Convert A$,"No"
  endif
  Convert B$,"Subtract background (",A$,") >"
  getvar A$,B$
  strpos "Y",A$
  if  value>0 then
    dosubref=1
    value=-1
    while value<0
      if ref_spec=0 then
        Convert A$,"Base"
      else
        if ref_spec<0 then
          Convert A$,"Apex"
        else
          Convert A$,"Ref"
```

contd. ...

```
        endif
      endif
      Convert B$,"Type of background, Ref/Base/Apex (",A$,") >"
      getvar A$,B$
      strpos "BA",A$
      if value>0 then
        ref_spec=0
      else
        strpos "AP",A$
        if value>0 then
          ref_spec=-1
        else
          strpos "RE",A$
          if value>0 then
            if ref_spec<0 then
              ref_spec=0.01
            endif
            convert A$,"Give time of reference spectrum (",ref_spec:7:3,") >"
            Getvar ref_spec,A$
          else
            mess "unknown type"
          endif
        endif
      endif
    endwhile
  else
    dosubref=-1
  endif
   CHECKWAVE
  Convert A$,"Wavelength range from (",st_wave:0:1,") >"
  Getvar st_wave,A$
  Convert A$,"Wavelength range to (",en_wave:0:1,") >"
  Getvar en_wave,A$
  if do smooth=1 then
    Convert A$,"Yes"
  else
    Convert A$,"No"
  endif
  Convert B$,"Smooth before search (",A$,") >"
  getvar A$,B$
  strpos "Y",A$
  if  value>0 then
```

contd. ...

```
    dosmooth=1
    Convert A$,"Number of smooth points, >5 (",smoothfac:0:0,") >"
    Getvar smoothfac,A$
  else
    do smooth=0
  endif
  Convert A$,"Match threshold for library (",SearThres:0:0,") >"
  Getvar SearThres,A$
  if dopeakpur=1 then
    Convert A$,"Yes"
  else
    Convert A$,"No"
  endif
  Convert B$,"Peak purity check (",A$,") >"
  getvar A$,B$
  strpos "Y",A$
  if  value>0 then
    dopeakpur=1
    Convert A$,"Match threshold for purity (",purThres:0:0,") >"
    getvar purthres,A$
  else
    dopeakpur=0
  endif
  if doprint=1 then
    Convert A$,"Yes"
  else
    Convert A$,"No"
  endif
  Convert B$,"Printout of results (",A$,") >"
  getvar A$,B$
  strpos "Y",A$
  if value>0 then
    doprint=1
  else
    doprint=0
  endif
  RETURN
```

contd. ...

```
!-----------------------------------!
! print heading                     !
!-----------------------------------!
NAME PRHEAD
    tof
    writeln 701,""
    write   701,"
    writeln 701,"C&P automated Library search macro (rev 4.06)#27&d@#15  ------"
!   writeln 701,"#10"
!   screenprint 640,500,printer:
    writeln 701,"#10"
    writeln 701,"Library file          : ",FILE_L$
    writeln 701,"        name          : ",lib_name$,#10
    writeln 701,"Match threshold       : ",Searthres:0:0
    if cor_sh>0 then
      writeln 701,"WL calibration cor.   : ",cor_sh:0:1," nm"
    endif
    write 701,"reference spectrum    : "
    if dosubref=1 then
      if ref_spec= then
        writeln 701, "Baseline"
      else
        if ref_spec<0 then
          writeln 701,"Apex"
        else
          writeln 701,ref_spec:0:2," min"
        endif
      endif
    else
      writeln 701,"None"
    endif
    if der_order>0 then
      writeln 701,"Order of derivative   : ",der_order:0:0
    endif
    write 701,"Smooth                : "
    if dosmooth=1 then
      writerealn 701,smoothfac:0:0
    else
      writeln 701,"None"
    endif
     CHECKWAVE
    writeln 701,"Wave range            : ",st_wave:0:1," to ",en_wave:0:1," nm"
    write 701,"Time window           : "
    if leftwin<0 then
```

contd. ...

```
      write 701,"None"
    else
      writeReal 701,leftwin:0:1
    endif
    if leftwin=rightwin then
    else
      write 701," to "
      if rightwin<0 then
        write 701,"None"
      else
        writeReal 701,rightwin:0:1
      endif
     endif
     writeln 701," %"
!   writeln 701,"Absorbance threshold  : ",ab_thres:0:1," %"
    write 701,"Peak purity threshold : "
    if dopeakpur=1 then
      writeRealn 701,purthres:0:0
    else
      writeln 701,"none"
    endif

  writeln 701,"#10#10File    : ",file_d$
  writeln 701,"Date    : ",inj_date$
  writeln 701,"Name    : ",data_name$
  writeln 701,"Info    : ",misc_info$
  writeln 701,"Operator: ",operator$
  if inj_num>0 then
    writeln 701,""
    write   701,"Sequence index : "seq_index:0:0
    writeln 701,"  vial : ",bottle:0:0," replicate : ",inj_num:0:0
  endif

  write   701,"#10#10------ Sample ------     #27&dD#14Match#27&d@#15"
  write   701,"     ----------------"
  writeln 701," Library ----------------------"
  writeln 701,"Peak#-----------------------------------------------"
  writeln 701,"---------------------------"
  RETURN !PRHEAD!
```

contd. ...

```
!-----------------------------------!
! ads a spectrum to x register      !
!-----------------------------------!
NAME AD_SPEC
  if ret_time<start_time then
    clear x
  else
    if ret_time>end_time then
      clear x
    endif
  endif
  gets header
  if nobjects=0 then
    ro -1
  else
    Convert Z$,header$
    gets header,y
    if nobjects=0 then
      ro -1
       ex x,t
    else
      i=1
      while i<=nobjects
        gets header,y,i
        strpos header$,Z$
        if value=1 then
          if len1=len2 then
            value=0
            i=nobjects
          else
            value=1
          endif
        else
          value=1
        endif
        i=i+1
      endwhile
      if value=0 then
        ro -1
      else
        merge
      endif
    endif
  endif
  RETURN
```

contd. ...

```
!------------------------------------!
! get up/top/down spectra            !
!------------------------------------!
NAME PEAK_SPEC
  cl x
  Spec Crest-(Peak_Width)/2,st_wave:en_wave
   AD_SPEC
  Spec Crest,st_wave:en_wave
   AD_SPEC
  Spec Crest+(Peak_Width)/2,st_wave:en_wave
   AD_SPEC
  RETURN !PEAK_SPEC!

!------------------------------------!
! find baseline spectrum.            !
!------------------------------------!
NAME GETBSPEC
  spec crest,st_wave:en_wave
  found=0
  while found=0
    if value=69 then
      ref_time=ret_time
      found=1
    else
      rol -1
      if scan_num>=lastspec then
        mes "No base spectrum found, set to:",ref_time,0,-5
        spec ref_time,st_wave:en_wave
        found=1
      else
        scan scan_num+1,st_wave:en_wave
      endif
    endif
  endwhile
  RETURN !GETBSPEC!
```

contd. ...

```
!-----------------------------------!
! subtract ref spec                 !
!-----------------------------------!
NAME SUBREF
  if ref_spec=0 then
     GETBSPEC
  else
    if ref_spec<0 then
      mul -1
      Spec Crest,st_wave:en_wave
      mul -1
    else
      Spec ref_spec,st_wave:en_wave
      ref_time=ret_time
    endif
  endif
  subtr
  SCREENPRINT 640,500

  RETURN !SUBREF!

!-----------------------------------!
! subtract ref spec                 !
!-----------------------------------!
NAME PRSEAR
  DADlib RES,1,x,n_object
  if entry>0 then
    il=1
    doit=1
    while doit>0
      DADlib RES,il,x,n_object
      doit=0
      if entry>0 then
        DAdlib Read,entry
        rol -1
        if il=1 then
          doit=1
        endif
        if match>searthres then
          doit=1
        endif
      endif
      if doit>0 then
        if il=1
```

contd. ...

```
        gets header,x,n_object
        strpos "(",header$
        Convert time,header$[3:(value-1)]
        if upslope=1 then
          writeln 701,""
          write 701,time:11:2,"        up"
        else
          if downslope=1 then
            write 701,time:11:2,"      down"
          else
            write 701,"     "
          endif
        endif
      else
        write 701,"                      "
      endif
      write 701,match:07:0,Ret_time:10:2,entry:5:0

      convert A$,"    "
      if match>searthres then
        if i1=1 then
          Convert comp_name$,"#27&dD#14",comp_name$,"#27&d@#15"
          if upslope=1 then
            convert A$," * "
          endif
          if downslope=1 then
            convert A$," * "
        endif
      endif
    endif
      writeln 701,A$,comp_name$
    endif
    i1=i1+1
  endwhile
endif
RETURN
```

contd. ...

```
!----------------------------------!
!  get derivative/smooth/norm spectra  !
!----------------------------------!
NAME SMDERNORM
  if der_order>0 then
    il=1
    while il<=der_order
      derivative
      il=il+1
    endwhile
  else
    if dosmooth=1 then
      smooth smoothfac
    endif
  endif
  norm 100
  RETURN !SMDERNORM!
!----------------------------------!
! ABORT                            !
!----------------------------------!
Name lib_ABORT
  mes A$,,0,-5
  if seq_run=yes then
    RETURN                   ! return to caller
  endif
                             ! stop

!----------------------------------!
! initializes the macro            !
!----------------------------------!
NAME INITAULIB
  dosubref=-1
  ref_spec=-1         ! no reference spectrum
  der_order=0         ! order of derivative
  dopeakpur=1         ! peak purity check (1=yes 0=no)
  adjanot=1           ! adjust compound nam
  smoothfac=11        ! smooth factor
  doprint=1           ! printout of results (1=yes, 0=no)
  fieldsize=15        ! annotation field size
  charsize=13         ! annotation character size
  anotrot=90          ! annotation string rotation
  sigtowin=1
  spetowin=2
  purtowin=4
```

contd. ...

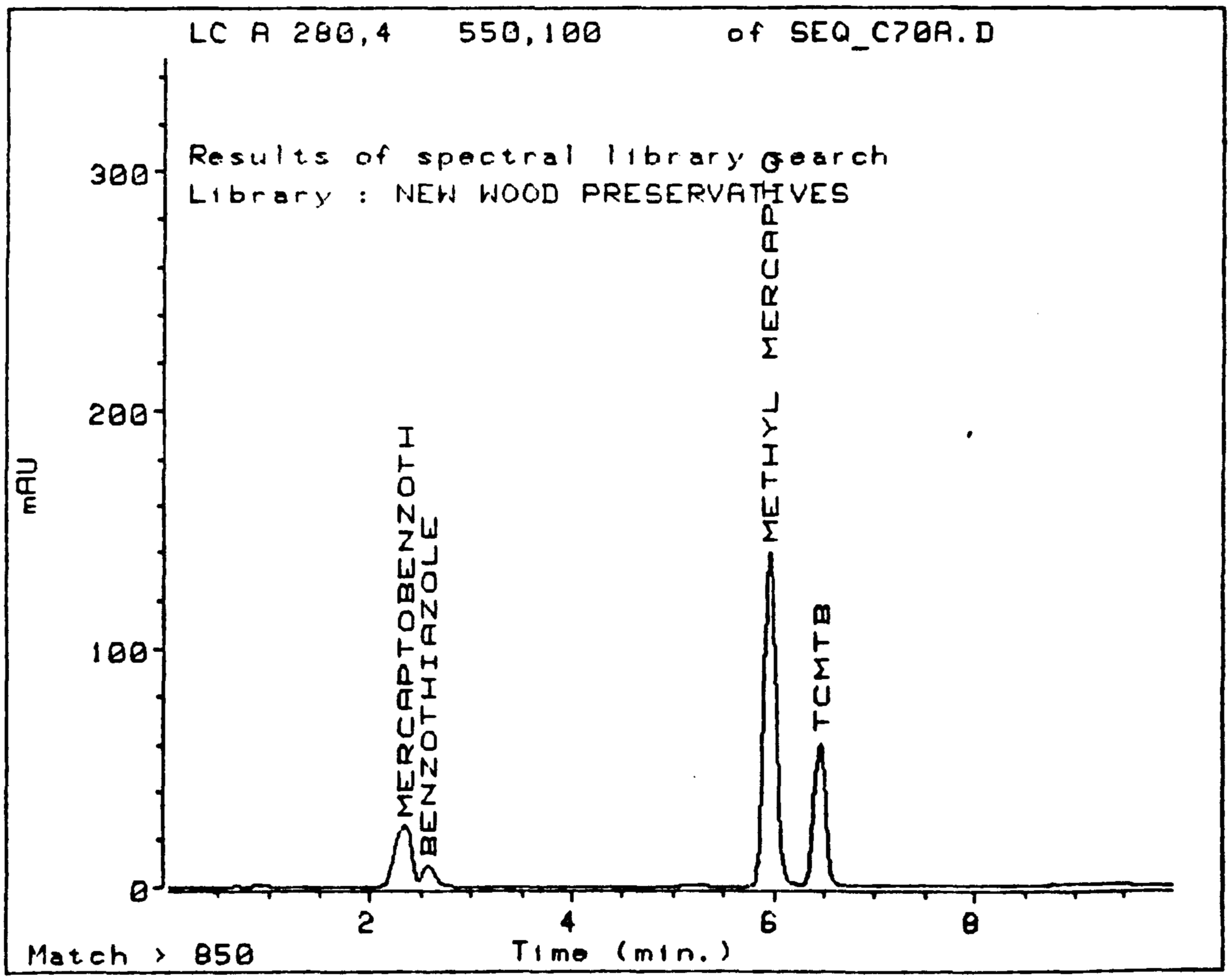
```
  seqinf
  if seq_run=0 then     ! Take the values from DATA EDITOR
    purthres=matchthres! purity threshold (above this threshold peak is pure)
    leftwin=leftwin     ! search window size 5 % of rete
    st_wave=startwave   ! start wavelength range
    en_wave=endwave     ! end wavelength range
    cor_sh=waveshift    ! wavelength calibration shift for spectral library
    ab_thres=absthres   ! mAU threshold for spectral library
    searthres=matchthres ! Match factor above which peak is identified.
  else                  ! set values
    purthres=500        ! purity threshold (above this threshold peak is pure)
    leftwin=20          ! search window size 5 % of retention time
    rightwin=15         ! when <0 then no search window
    st_wave=0           ! start wavelength range
    en_wave=0           ! end wavelength range
    cor_sh=0            ! wavelength calibration shift for spectral library
    ab_thres=2          ! mAU threshold  peak is identified.
    searthres=500       ! Match factor above which peak is identified.
  endif

  Convert AutolibIn$,"AUTOLIB 4.06 Initialized"  ! set autolib is initialized.

  RETURN

!--------------------------------------!
! removes the macro                    !
!--------------------------------------!
NAME REMUSERMAC
  RemAUTOLIB,MakePrint,MakePlot,InitAuLib,CheckWave,SetVar,PrHead,Ad_Spec
  Rem Peak_SPec,GetBspec,SubRef,PrSear,SmDerNorm,Lib_Abort
  RETURN

!--------------------------------------!
!                                      !
!--------------------------------------!
NAME DOUSERMAC
   VM
  OLD_VM=VM_MEM
  VM ,60000
   AUTOLIB
  VM ,OLD_VM
   DISPSTATUS=1
   REMUSERMAC
  RETURN
```

contd. ...

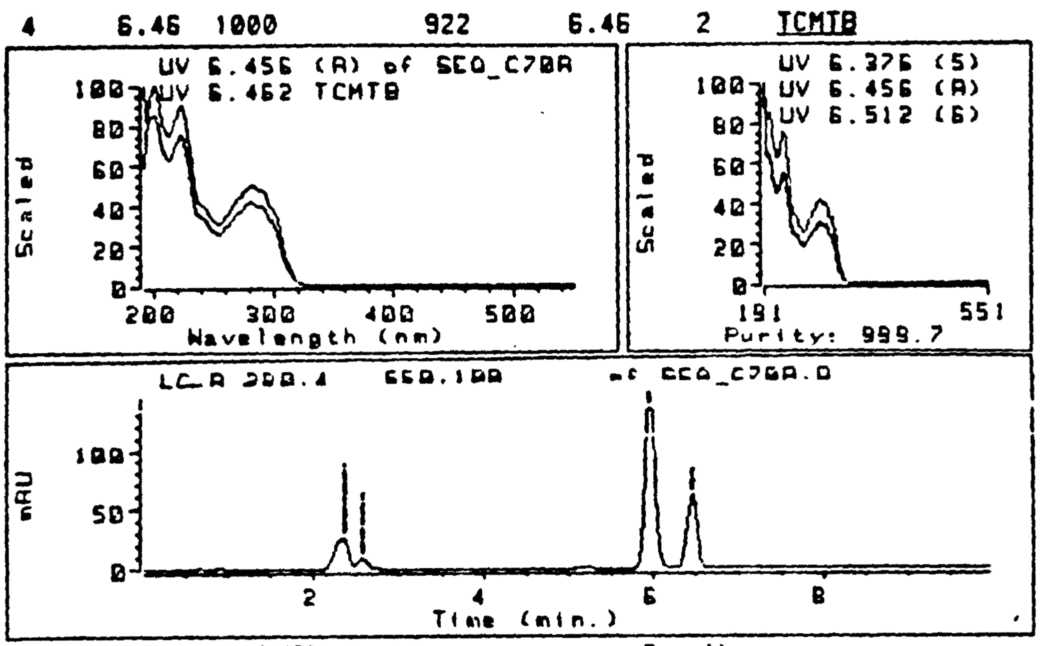
HPLC CHROMATOGRAM AND LIBRARY SEARCH RESULTS FROM A STANDARD MIX OF TCMTB AND METABOLITES:

AUTOMATED LIBRARY FINAL OUTPUT

Sample			Match	Library		
Peak#	Time	Purity		Time	Entry#	Name
1	2.34	871	999	2.24	3	MERCAPTOBENZOTHIAZOLE
2	2.57	1000	999	2.53	4	BENZOTHIAZOLE
3	5.96	1000	901	5.98	1	METHYL MERCAPTOBENZOTHIAZOLE
4	6.46	1000	922	6.46	2	TCMTB



AUTOMATED LIBRARY SEARCH MACRO



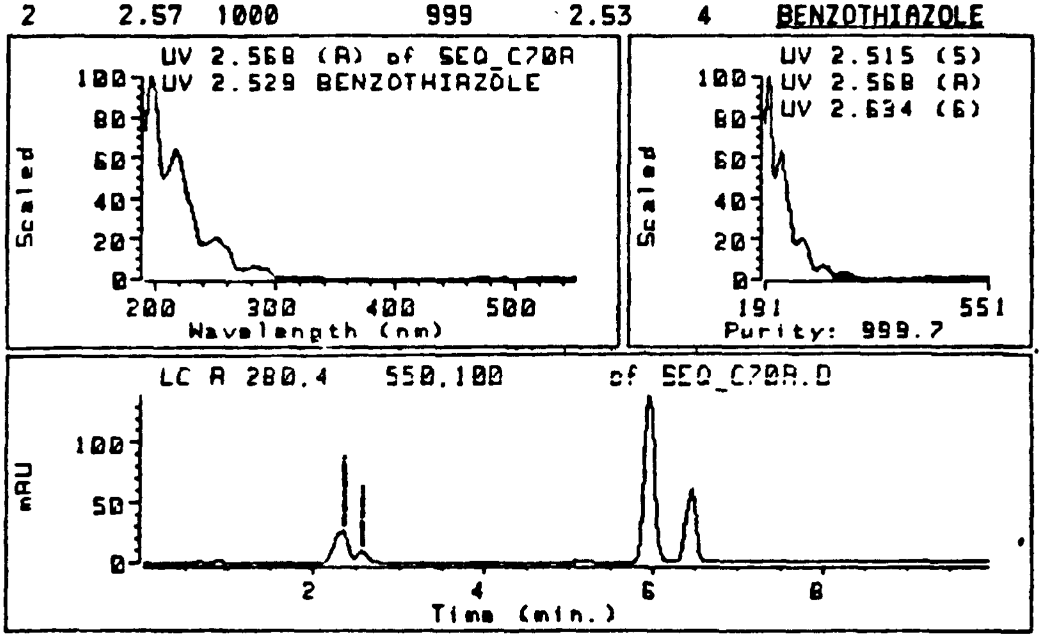
Peak# 4 time: 6.456
Entry#: 2.0000earch.high_Match: 922.48

T: null.
Z: LC A 280,4 550,100
Y: LC A 280,4 550,100
X: Set of 2 UV

TCMTB

AUTOMATED LIBRARY SEARCH MACRO

2 2.57 1000 999 2.53 4 BENZOTHIAZOLE



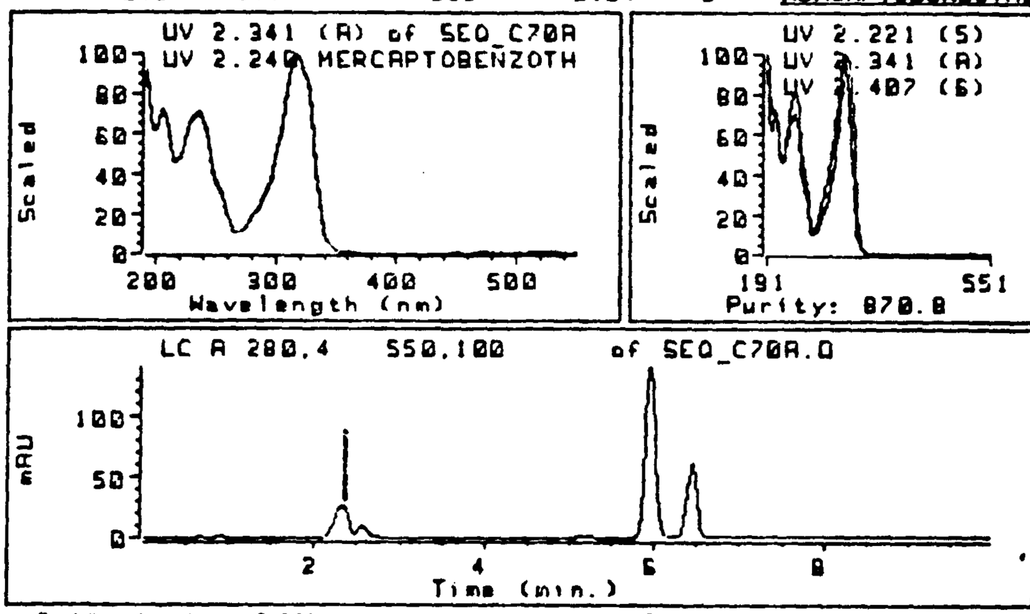
Peak# 2 time: 2.568
Peak spectra search.....

T: Set of 2 UV
Z: null).
Y: LC A 280,4 550,100
X: LC A 280,4 550,100

Benzothiazole

AUTOMATED LIBRARY SEARCH MACRO

Sample Peak#	Sample Time	Sample Purity	Match	Library Time	Library Entry#	Library Name
1	2.34	871	999	2.24	3	MERCAPTOBENZOTHIAZOLE



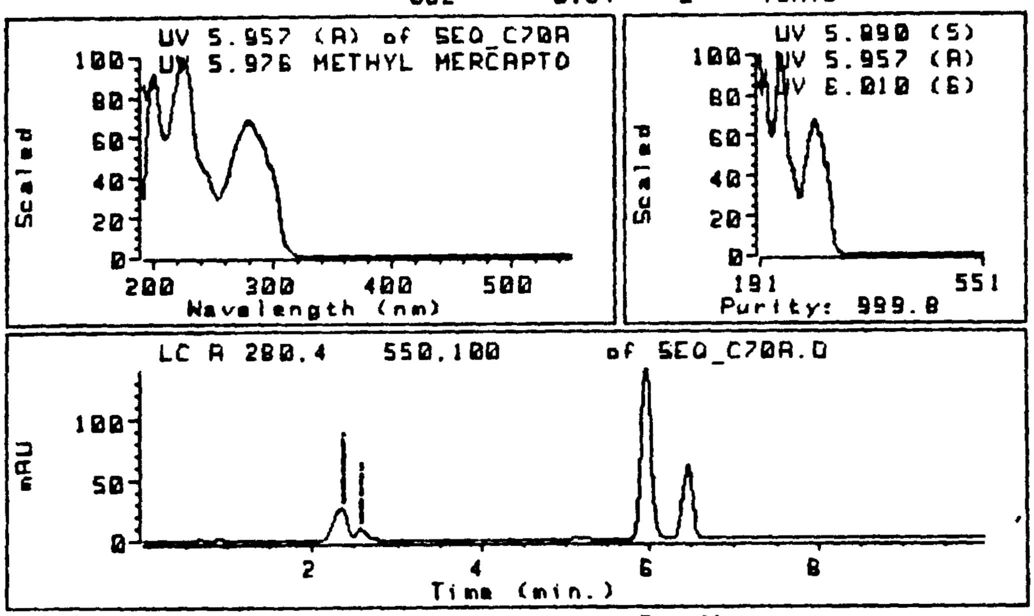
Peak# 1 time: 2.341
Entry#: 3.000Search.high_Match: 998.52

T: null.
Z: LC A 280,4 550,100
Y: LC A 280,4 550,100
X: Set of 2 UV

2-Mercaptobenzothiazole

AUTOMATED LIBRARY SEARCH MACRO

3	5.96	1000	901	5.98	1	METHYL MERCAPTOBENZOTHIAZOLE
			862	5.84	2	TCMTB



Peak# 3 time: 5.957
Entry#: 1.0000earch.high_Match: 900.73

T: null.
Z: LC A 280,4 550,100
Y: LC A 280,4 550,100
X: Set of 2 UV

2-Methylmercaptobenzothiazole