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CHLOROPHENOL LEVELS IN AQUATIC LEECHES FROM  
SELECTED SITES IN SOUTHWESTERN NEW BRUNSWICK

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## EXECUTIVE SUMMARY

### **Chlorophenol Levels in Aquatic Leeches from Selected Sites in Southwestern New Brunswick**

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**Environmental Contaminants Division**  
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When we observed that leeches in Canagagigue Creek were accumulating chlorophenols to concentrations orders of magnitude higher than other aquatic organisms, we proposed that these organisms be investigated as potential biointegrators of aquatic contaminants. In order to determine whether this behaviour was general, leeches in two river systems in southwestern New Brunswick were collected and analysed for chlorophenols and other organic contaminants. Site selection was based on proximity to a known source of organic pollution or to a NAQUADAT station. The five sites sampled include three sites on the St. John and one site each on the Oromocto and St. Croix Rivers. Seven species of leech were found with the dominant species being *E. punctata*, *H. stagnalis* and *G. complanata*. Leeches were found to be good indicators of chlorophenol contamination. Only three chlorophenols were detected in the water but a total of twelve were detected in leeches from the various sites. Concentrations in the leeches were from three to four orders of magnitude higher than the water concentrations. Results for neutral organochlorine contaminants were and were difficult to interpret. Further study of the accumulation of these contaminants in leeches is required.

## MANAGEMENT PERSPECTIVE

The potential use of biological techniques to augment sampling of water and sediment for assessment of water quality is an area of active interest for IWD. One such technique involves the use of aquatic organisms as integrators of contaminants. This technique would provide more representative data in cases where sources of contaminants were sporadic. In addition, when contaminant concentrations in the water are below detection limits, biota samples can be used to screen for the presence of these compounds. In order for these techniques to be of use in a monitoring program, suitable species need to be identified. These species should be widely distributed and should readily concentrate the contaminants of interest. This paper examines the levels of chlorophenols in leeches from southwestern New Brunswick. More chlorophenols were detected in leeches than in water, confirming that leeches readily concentrate these contaminants. Neutral organochlorine results for the same leeches were more difficult to interpret, indicating that more work needs to be done before a comprehensive monitoring program involving leeches could be justified.

## RÉSUMÉ ADMINISTRATIF

Niveaux de chlorophénols relevés dans les sanguines prélevées aux sites d'étude dans le sud-ouest du Nouveau-Brunswick

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Lorsque nous avons constaté que les sanguines du ruisseau Canagagigue accumulaient les chlorophénols à des niveaux qui dépassaient plusieurs fois ceux des autres organismes aquatiques, nous avons proposé d'évaluer la possibilité d'utiliser les sanguines comme indicateurs biologiques des contaminants aquatiques. Pour vérifier si toutes les sanguines agissent comme des bioaccumulateurs, nous avons recueilli des spécimens dans deux bassins du sud-ouest du Nouveau-Brunswick et avons analysé leur charge corporelle en chlorophénols ainsi que d'autres contaminants organiques. Les sites ont été choisis en fonction de leur proximité d'une source d'effluents connue ou d'une station NAQUADAT. Trois des cinq sites se situaient sur la rivière Saint-Jean tandis que les deux autres étaient sur les rivières Oromocto et Sainte-Croix respectivement. Nous y avons trouvé sept espèces de sanguines dont les plus répandues étaient E. punctata, H. stagnalis et G. complanata. Les sanguines se sont révélées d'excellents indicateurs du niveau de contamination par les chlorophénols. Bien que nous n'ayons détecté que trois chlorophénols dans l'eau, nous en avons relevé douze dans les sanguines en provenance des différents sites. Les charges corporelles des sanguines étaient trois ou quatre fois plus élevées que les concentrations dans l'eau ambiante. Il a été difficile d'interpréter les résultats obtenus pour les contaminants organochlorés neutres. Il faudra donc poursuivre les recherches portant sur la bioaccumulation de ces derniers chez les sanguines.

## PERSPECTIVE-GESTION

La Direction générale des eaux intérieures s'intéresse activement à la possibilité d'employer des techniques biologiques pour agrandir la palette des échantillons d'eau et de sédiments afin de déterminer la qualité de l'eau. L'une de ces techniques consiste à étudier la bioaccumulation des contaminants chez les organismes aquatiques. Cette technique d'analyse produirait des résultats plus stables et représentatifs lorsque les apports de contaminants sont intermittents. De plus, si les concentrations des contaminants chutent sous le seuil de détection, on peut tout de même repérer les composés à surveiller en analysant les échantillons du biote. Pour pouvoir appliquer cette technique d'étude dans le cadre d'un programme de contrôle des contaminants, il faut connaître les espèces convenables. Dans ce rapport, on fait état des charges corporelles de chlorophénols relevées dans les sanguines du sud-ouest du Nouveau-Brunswick. Etant donné qu'on a constaté dans les sanguines des teneurs en chlorophénols plus élevées que dans l'eau, on a pu confirmer que ces organismes accumulent les contaminants du milieu ambiant. Cependant, les mesures des composés organochlorés neutres chez les mêmes sanguines ont donné des résultats moins évidents. Il y aurait donc lieu de perfectionner la méthode avant que l'on puisse justifier son emploi dans le cadre d'un programme global de contrôle des contaminants.

### ABSTRACT

When we observed that leeches in Canagagigue Creek were accumulating chlorophenols to concentrations orders of magnitude higher than other aquatic organisms, we proposed that these organisms be investigated as potential bioindicators of aquatic contaminants. In order to determine whether this behaviour was general, leeches in two river systems in southwestern New Brunswick were collected and analysed for chlorophenols and other organic contaminants. Site selection was based on proximity to a known source of organic pollution or to a NAQUADAT station. The five sites sampled include three sites on the St. John and one site each on the Oramocto and St. Croix Rivers. Seven species of leech were found with the dominant species being *E. punctata*, *H. stagnalis* and *G. complanata*. Leeches were found to be good indicators of chlorophenol contamination. Only three chlorophenols were detected in the water but a total of twelve were detected in leeches from the various sites. Concentrations in the leeches were from three to four orders of magnitude higher than the water concentrations. Results for neutral organochlorine contaminants were less satisfactory and were difficult to interpret. Further study of the accumulation of these contaminants in leeches is required.

## RÉSUMÉ

Lorsque nous avons constaté que les sanguines du ruisseau Canagagigue accumulaient les chlorophénols à des niveaux dépassant plusieurs fois ceux des autres organismes aquatiques, nous avons proposé d'évaluer la possibilité d'utiliser ces organismes comme indicateurs biologiques des contaminants aquatiques. Pour vérifier si toutes les sanguines agissent comme des bioaccumulateurs, nous avons recueilli des spécimens dans deux bassins du sud-ouest du Nouveau-Brunswick et avons analysé leur charge corporelle en chlorophénols ainsi que d'autres contaminants organiques. Les sites ont été choisis en fonction de leur proximité d'une source d'effluents connue ou d'une station NAQUADAT. Trois des cinq sites se situaient sur la rivière Saint-Jean tandis que les deux autres étaient sur les rivières Oromocto et Sainte-Croix respectivement. Nous y avons trouvé sept espèces de sanguines dont les plus répandues étaient E. punctata, H. stagnalis et G. complanata. Les sanguines se sont révélées d'excellents indicateurs du niveau de contamination par les chlorophénols. Bien que nous n'ayons détecté que trois chlorophénols dans l'eau, nous en avons relevé douze dans les sanguines en provenance des différents sites. Les charges corporelles des sanguines étaient trois ou quatre fois plus élevées que les concentrations dans l'eau ambiante. Il a été difficile d'interpréter les résultats obtenus pour les contaminants organochlorés neutres. Il faudra donc poursuivre les recherches portant sur la bioaccumulation de ces derniers chez les sanguines.

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### INTRODUCTION

Surveillance programs to monitor levels of environmental contaminants in aquatic systems frequently make use of only water samples. A more complete picture of environmental contamination could be obtained by analyzing biological indicator organisms - in this case, referring to organisms having a high bioaccumulation potential for the compound(s) in question. Such analyses can indicate the presence of compounds whose water concentrations are below detection limits and whose presence therefore go undetected in conventional water sampling. In addition, analysis of indicator organisms can give an indication of the bioavailability of the compounds of interest.

Freshwater leeches from an industrially polluted stream in southern Ontario have been shown to accumulate very high levels of chlorophenols when compared with residues found in many other aquatic organisms.<sup>1</sup> We have therefore suggested leeches for consideration as bioindicators of aquatic organic contamination.

The present study examines the levels of chlorophenols and some selected neutral organochlorine contaminants in leeches from selected sites in southwestern New Brunswick. The relationship between levels of these contaminants in leeches and water is examined and the bioaccumulation capacities of several leech species is compared.

## METHODS

### Site Location

Sites were selected based on proximity to a source of pollution and/or a NAQUADAT Station. The sites are shown on the map (Fig. 1). The St. Croix site, at Upper Mills, is about 8 km downstream from a large pulp and paper mill at Woodland, Maine, which discharges into the St. Croix River. The nearest NAQUADAT station (#00NB 01AR 0001) is 4 km downstream at Milltown. The Oromocto site is on the north branch of the Oromocto River in the village of Tracy (pop. 662<sup>3</sup>), and is NAQUADAT station #00NB 01AM 0001. The other three sites are all on the Saint John River. The Nashwaak site is downstream from Fredericton (pop. 45,248<sup>3</sup>) below the confluence of the Saint John and Nashwaak Rivers. The nearest NAQUADAT station (#00NB 01AL 0007) is on the Nashwaak R. just upstream from the confluence. The Nackawic site is immediately below the outfall from the St. Anne-Nackawic Pulp and Paper Co., a major mill (employment for 1980 - 425 to 450) which produces bleached (kraft) hardwood wood pulp. This site, which is at the confluence of the Saint John and Nackawic

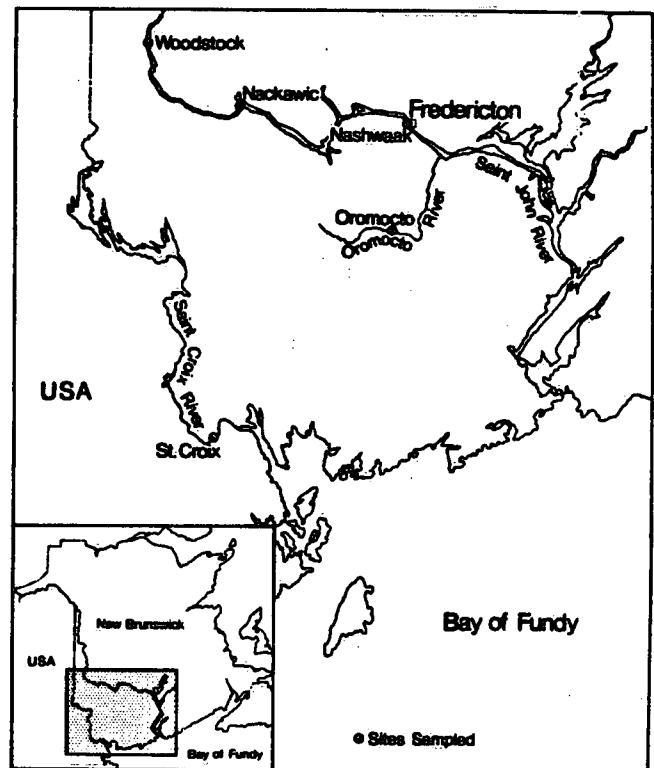


Figure 1 LOCATIONS OF STUDY SITES

Rivers is NAQUADAT station #00NB 01AK 0009. The Woodstock site  
is downstream from the town of Woodstock (pop. 4869<sup>3</sup>) in a major  
potato farming and processing area. NAQUADAT station  
#00NB 01AJ 0014 is about 4 km upstream.

#### Sample Collection

Water and leeches were collected from the St. Croix, Oromocto and Nashwaak sites on Oct. 12, 1982 and from the Nackawic and Woodstock sites on the following day. Two 1L water samples were collected at each site. Five pellets of sodium hydroxide were added to one sample and 50 mL. methylene chloride to the other as preservatives for the chlorophenol and neutral organochlorine analyses respectively. Leeches were hand picked from the undersides of rocks and other smooth-surfaced objects in shallow areas along the rivers' shorelines. They were collected in glass jars and transported on ice to the laboratory where they were held overnight at 4 C. The following morning they were identified and sorted live. Samples of each species were weighed wet to the nearest 1 mg. and dissolved in about 7 mL. of concentrated hydrochloric acid for storage prior to analysis.

#### Analytical Methods

##### Water

The water samples that had been preserved with base were analysed for chlorophenols using a modification of the method of Chau and Coburn.<sup>4</sup> After adjustment of the pH to about 2 with concentrated hydrochloric acid, the samples were triple extracted with a total of 100 mL. pesticide grade toluene (40 + 30 + 30 mL.). The chlorophenols were back extracted with 0.1 M potassium

carbonate and derivatized with acetic anhydride. A 25 m x 0.21 mm ID fused silica column coated with OV1 and an electron capture detector were used for the gas chromatographic analysis of the chlorophenol acetates. The oven temperature was programmed from 90° C to 260° C at 4 deg/min.

The second water sample from each site was analysed for neutral organochlorines. The methylene chloride that had been added in the field was separated and the aqueous layer was extracted twice more with 40 mL. of solvent. The combined extracts were dried with sodium sulfate and, after the addition of 10 mL. of pesticide grade iso-octane, evaporated to 1 mL. The extract was analysed by gas chromatography without further clean-up.

#### Leeches

The leeches, already dissolved in concentrated hydrochloric acid, were extracted with 100 mL. of pesticide grade toluene (40 + 30 + 30 mL.). The toluene layer was washed with 50 mL. organic free water, extracted with 0.1 M potassium carbonate (40 + 30 + 30 mL.) and the carbonate fraction was analysed for chlorophenols as above. The toluene fraction was then washed with 50 mL. of organic free water, dried by passing it through a bed of anhydrous sodium sulfate and evaporated to a final volume of 1 mL. The extract was then analysed for organochlorines by gas chromatography without further clean-up.

## RESULTS AND DISCUSSION

### Chlorophenols

The concentrations of chlorophenols in water and leeches from all five sites are presented in Table I. In the water samples, individual chlorophenols ranged from not detectable to 0.035 ug/L. Three chlorophenols, 2,4,6-trichlorophenol (2,4,6-TCP), 2,3,4,6-tetrachlorophenol (2,3,4,6-TTCP) and pentachlorophenol (PCP) were detected in water from Nackawic which had the highest levels of the five sites. Lower levels of 2,4,6-TCP were detected in water from the St. Croix site and from Woodstock; PCP was also present at Woodstock. No chlorophenols were found in water from either Oromocto or Nashwaak.

In contrast to the three chlorophenols detected in the water, a total of twelve chlorophenol isomers were found in leeches from the five sites. The major chlorophenols observed in the leeches were the same three detected in the water although 2,4-dichlorophenol (2,4-DCP) and 2,3,4,5-tetrachlorophenol (2,3,4,5-TTCP) were also present at significant levels in leeches from some sites. Previous work on the bioaccumulation of chlorophenols in leeches revealed considerable differences between species.<sup>1</sup> Such differences must therefore be considered when comparing levels in leeches from various sites. Table II presents site to site comparisons of the five major chlorophenols for four leech species. The data show that Nackawic is the most contaminated site followed by Woodstock and St. Croix with similar levels. Levels of chlorophenols were lowest in leeches from Oromocto and Nashwaak. This relative ranking correlates

TABLE I. Chlorophanol concentrations in leeches and water from the five study sites.

TABLE II. Comparison of the tissue residues of the five major chlorophenols in four leech species.

| Species:                         | Site | Chlorophenol residues (µg/g) |           |              |              | PCP |
|----------------------------------|------|------------------------------|-----------|--------------|--------------|-----|
|                                  |      | 2,4-DCP                      | 2,4,6-TCP | 2,3,4,6-TTCP | 2,3,5,6-TTCP |     |
| <i>Halobdella stagnalis</i> :    |      |                              |           |              |              |     |
| Nachawic                         | 120  | 150                          | 550       | 80           | 650          |     |
| St. Croix                        | 120  | 70                           | 160       | 260          | 35           |     |
| Woodstock                        | 140  | 120                          | 350       | 30           | 120          |     |
| Oronocto                         | 50   | 20                           | 15        | 20           | 30           |     |
| <i>Exeipodella punctata</i> :    |      |                              |           |              |              |     |
| St. Croix                        | 80   | 450                          | 310       | 20           | 40           |     |
| Woodstock                        | 80   | 350                          | 240       | 10           | 20           |     |
| Nashwaak                         | 30   | 35                           | 30        | 10           | 20           |     |
| Oronocto                         | nd   | 25                           | nd        | nd           | 5            |     |
| <i>Glossiphonia complanata</i> : |      |                              |           |              |              |     |
| Nachawic                         | 30   | 40                           | 60        | 5            | 25           |     |
| St. Croix                        | nd   | 50                           | 35        | nd           | nd           |     |
| Nashwaak                         | nd   | 15                           | nd        | 25           | 30           |     |
| <i>Dina parva</i> :              |      |                              |           |              |              |     |
| Woodstock                        | 150  | 180                          | 15        | nd           | 25           |     |
| Oronocto                         | 25   | 100                          | 5         | nd           | 10           |     |

well with the water data.

As mentioned, leeches may vary in their bioaccumulation potential from species to species. In the present study, *G. complanata* accumulated the lowest levels - 60 ug/L or less even at the most contaminated site. Although there is not enough data to draw a definite conclusion, the inter-species comparison reveals an interesting feature. In contrast to *H. stagnalis* which had higher levels of the more highly chlorinated phenols, both *E. punctata* and *D. parva* had higher levels of the lower chlorinated isomers. The reason for this pattern may be that the latter two species are capable of dechlorinating chlorinated phenols and *H. stagnalis* is not. Such behaviour is not without precedent. In a study of the dehydrochlorination of DDT by Malaysian freshwater leeches, Kimura et al noted that some species were capable of this reaction while others were not.<sup>5</sup>

*H. stagnalis* appears to be the best bioindicator on the basis of its high bioaccumulation potential for chlorophenols. This species is common and widespread in North America so it would also be considered the best species for comparing geographically separate areas. It was the only species found at all five sites in this study (including Nashwaak - this sample was lost during analysis). In a survey of 13 sites along the Grand River, Ontario and its tributaries, *H. stagnalis* was found at all 13 sites and *E. punctata* at 11 (to be published).

#### Neutral Organochlorines

The results of the neutral organochlorine analyses are presented in Table III. The following neutral organochlorines

TABLE III. Organochlorine concentrations in leeches and water from the five study sites.

| Sample                            | # Indiv. | Wet wt. (g) | Organochlorine concentrations (mg/g for leeches; ug/L for water) |           |             |       |          |
|-----------------------------------|----------|-------------|--|-----------|-------------|-------|----------|
|                                   |          |             | 1,2-DCB  | 1,2,3-TCB | 1,2,3,4-TCB | HCB   | P,P'-DDT |
| <i>Halobdella stagnalis</i>       | 15       | .147        | nd   | nd        | nd          | nd    | nd       |
| <i>Glossiphonia complanata</i>    | 29       | 1.147       | nd   | nd        | nd          | nd    | nd       |
| Water                             |          |             | .070   | .021      | .023        | .001  | nd       |
| <i>Halobdella stagnalis</i>       | 29       | .371        | 50   | nd        | nd          | 15    | 45       |
| <i>Erpobdella punctata</i>        | 4        | .260        | nd   | nd        | nd          | nd    | nd       |
| <i>Glossiphonia complanata</i>    | 7        | .402        | nd   | nd        | nd          | nd    | 16       |
| Water                             |          |             | .060   | .023      | nd          | nd    | .035     |
| <i>Halobdella stagnalis</i>       | 23       | .156        | nd   | nd        | nd          | nd    | 150      |
| <i>Erpobdella punctata</i>        | 2        | .268        | nd   | nd        | nd          | nd    | 45       |
| <i>Diplo parva</i>                | 1        | .198        | nd   | nd        | nd          | nd    | 50       |
| Water                             |          |             | .030   | nd        | nd          | nd    | nd       |
| <i>Erpobdella punctata</i>        | 6        | .665        | nd   | nd        | nd          | nd    | 30       |
| <i>Glossiphonia complanata</i>    | 19       | .157        | nd   | nd        | nd          | nd    | 80       |
| <i>Halobdella fusca</i>           | 13       | .693        | 40   | nd        | nd          | nd    | 10       |
| Water                             |          |             | .010   | nd        | nd          | <.001 | nd       |
| <i>Halobdella stagnalis</i>       | 22       | .558        | 60   | nd        | nd          | nd    | nd       |
| <i>Erpobdella punctata</i>        | 1        | .322        | nd   | nd        | nd          | nd    | nd       |
| <i>Diplo parva</i>                | 7        | .688        | nd   | nd        | nd          | nd    | nd       |
| Water                             |          |             | .030   | .003      | .003        | .003  | .010     |
| <i>Oncocrotalus</i> River - Tracy |          |             |  |           |             |       |          |

were found in the water; 1,2-dichlorobenzene at all five sites, 1,2,3-trichlorobenzene at all but Woodstock and Nashwaak, 1,2,3,4-tetrachlorobenzene at Nackawic and Oromocto, hexachlorobenzene at all but Woodstock and St. Croix, and p,p'-DDE at Oromocto and St. Croix.

Levels of organochlorines in leeches did not correspond well to levels in water. For example, p,p'-DDE was found in leeches from all sites but Oromocto where it was present in the water. The highest levels of this compound were found in leeches from Woodstock and Nashwaak where none was detected in the water. Hexachlorobenzene was found in leeches from St. Croix although none was detected in the water there. Conversely, at sites where hexachlorobenzene was present in the water, none was found in the leeches. It is evident from this data that the relationship between levels of neutral organochlorines in water and leeches is more complicated than that for chlorophenols. Factors which could complicate this relationship include the nature of the source (diffuse vs point), the temporal variation of the source (constant vs sporadic), the accumulation rates of the individual species for these contaminants and their ability to metabolize or excrete them.

#### ACKNOWLEDGEMENTS

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Zoology Division, Museum of Natural Sciences, National Museums Canada, identified the leech species.

REFERENCES

1. Metcalfe, J. L., M. E. Fox and J. H. Carey. 1984. Aquatic leeches (*Hirudinea*) as bioindicators of organic chemical contaminants in freshwater ecosystems. *Chemosphere*, 13: 143-150.
2. Inland Waters Directorate, Water Quality Branch, Environment Canada. 1979. *Water Quality Data, New Brunswick, 1961-1977*.
3. Department of Commerce and Development, Province of New Brunswick. 1981. *New Brunswick Manufacturers and Products, 1980*. 272 p.
4. Chau, A. S. Y., and J. A. Coburn. 1974. Determination of pentachlorophenol in natural and waste waters. *J. Assoc. Off. Anal. Chem.*, 57: 389-393.
5. Kimura, T., H. L. Keegan and T. Haberkorn. 1967. Dehydrochlorination of DDT by Asian blood-sucking leeches. *Amer. J. Trop. Med. Hyg.*, 16: 668-690.