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ECOTOXICITY OF HIGHWAY RUNOFF: PRELIMINARY BASELINE STUDIES

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ABSTRACT

In the Province of Ontario, in spite of recent improvements in point source controls through programs like MISA, in most urbanized areas it is impossible to attain Water Quality targets and restore beneficial uses without some control of stormwater and combined sewer overflow discharges.

Highways and associated land corridors may produce significant volumes of runoff conveying a variety of contaminants and since traditionally, the design of highway drainage focused on removing runoff to ameliorate traffic safety issues, the impact of dispersed highway runoff was rarely considered. To develop some appreciation of such impacts a preliminary study of the toxicity of highway runoff was conducted.

For this preliminary survey-type study of toxicant and genotoxicant presence in highway runoff, a section of the QEW highway (Burlington Skyway) was selected. Two runoff samples were collected, one within minutes of the onset of a heavy storm and another after approximately 15 hours of light rainfall. These samples were tested with a battery of bioassays to establish the presence of toxicants.

The bioassay data from these two road runoff samples suggest that the two sets of samples trigger responses in different bioassays, thus supporting the need for using the battery of tests approach. Also the data indicate that toxicants and genotoxicants may be present in all runoff events and phases of runoffs from this bridge site, i.e. beginning of a rain event and even after 15 hours of rain.

INTRODUCTION

In the Province of Ontario, in spite of recent improvements in point source controls through programs like MISA, most urbanized areas are unable to attain Water Quality targets and restore beneficial uses without some control of stormwater and combined sewer overflow discharges. This has been well documented Ontario Pollution Prevention and Control Planning Studies and in the development of Remedial Action Plans.

Highways and associated land corridors produce significant volumes of runoff conveying a variety of contaminants. Traditionally, the design of highway drainage focused on removing runoff to ameliorate traffic safety issues and to ensure that under design conditions, the width of runoff flow on the pavement (the so-called) gutter flow) would not exceed the design spread. Consequently, flow capacities and locations of individual highway drainage elements were designed to keep the width of runoff flow below the design spread (Marsalek, 1982).

In the mid 1980's the pollution potential of highway runoff was recognized and numerous studies of highway runoff chemistry were conducted. In North America the most extensive studies were conducted by the U.S. Federal Highway Administration (Versar, 1985; Woodward-Clyde, 1989).

Literature data (Marsalek, 1990; Mulliss et al., 1993; Jones et al., 1993; Borchardt, 1993) indicate that highway runoff conveys various contaminants in concentrations which may be acutely toxic or genotoxic to freshwater aquatic life. Traditional chemical testing suffers from a number of weaknesses such as; (a) it measures the chemical abundance rather than the bioavailability, (b) it deals with singular substances rather than with their integrated effects, (c) it cannot even hope to analyze for the great variety of chemicals which maybe/are present, and (d) the analytical process may be relatively slow and costly.

One generally accepted solution and support for the above problems is the use of ecotoxicological procedures i.e. bioassays. The main advantages of an ecotoxicological based approach are (a) an integrated assessment of the impacts of various bioavailable substances and (b) a relatively fast and inexpensive testing compared to the traditional chemical menu.

The ecotoxicological findings obtained from two different types of road runoff are described in this report.

METHODS

Sampling area and sample collection

For this preliminary survey-type study, a section of the QEW highway (Burlington Skyway) was

selected. This section of highway has several advantages in that it is not overly influenced by tracked-on mud/soil, the runoff is easily collected from the downspouts on the bridge without traditional roadside influences and because of the curbing we are fairly certain we are measuring only road runoff and ambient settled air pollution.

On June 27 about 15:00 hours , after about two weeks of hot dry weather, a sudden heavy rain started. Twenty minutes after the storm began we collected a water sample from the overhead downspout situated by the second support tower, north of the Burlington Canal. The second sample was collected on the morning of Aug. 3 after and during a moderate ongoing overnight rainfall. Thus we have a first flush sample and a sample after the road has been washed for 15 or more hours.

On Sept. 28 after an overnight rain, a soil sample was collected from a puddle immediately below a downspout and another sample was collected from a vacant field near the bridge, but not being impacted by the highway runoff. This field has been undisturbed for at least three years. The puddle mud sample was ≈ 50% water while the field sample had ⟨10% water content.

Bioassays

The June 27 and Aug 3 runoff water samples were subjected to the following bioassays: *Daphnia magna* acute toxicity test, reverse electron transport (RET) submitochondrial particle (SMP) assay, forward electron transport (FET) submitochondrial particle (SMP) assay, SOS-Chromotest without S9, SOS-Chromotest solid phase test, Microtox , *Panagrellus redivivus* assay, *Spirillum volutans* assay, seed germination test using Buttercrunch lettuce seeds (*Lactuca sativa),* (Dutka, 1995; Dutka et al. 1995; Dutka et al. 1994). The water samples were concentrated 10X by flash evaporation for all samples except *Daphnia magna.*

The soil samples were tested for acute toxicity using DSTTP procedure (Kwan 1990) and for genotoxicants the SOS-Chromotest solid phase test was used.

BIOASSAY RESULTS

1) Submitochondrial bioassays

	June 27	Aug. 3	
	1X	1X	10X*
SMP RET % inhibition	68	53	100
SMP FET % inhibition	41	33	81

10X*=concentrated 10 times (X) by flash evaporation.

2) *Panagrellus redivivus*

	June 27		Aug. 3	
	1X	10X	1X	10X
Nematode % survival	100	94	96	87
Nematode % growth	100	85	94	76
Nematode % maturation	97	67	78	28

3) Microtox

	June 27		Aug. 3	
	1X	10X	1X	10X
% sample producing EC_{50}	>100	58.8	>100	>100
% sample producing EC_{10}	>100	13.8	>100	>100

4) SOS-Chromotest Plate Version

	June 27		Aug. 3	
	1X	10X	1X	10X
Induction Factor	1.05	1.07	1.03	1.17

5) SOS-Chromotest Solid Phase Test

	June 27		Aug. 3	
	1X	10X	1X	10X
Genotoxicity CIP* value	11		2	4
Cytotoxicity CIP value	0		0	2

CIP*= colour Index Profile (Dutka et al. 1995)

6) *Daphnia magna*

	June 27	Aug. 3
	1X	1X

EC or % sample=	**EC_{50}**	**EC_{30}**	**100%**

7) *Spirillum volutans*

	June 27	**Aug. 3**
Concentration producing EC_{90}	**1.6X**	**2.5X**

8) Seed Germination & Root elongation

	June 27		**Aug. 3**	
	1X	**10X**	**1X**	**10X**
% inhibition	**0**	**100**	**0**	**100**

9) DSTTP

	Puddle mud	**Field soil**
% sample producing toxic effect	**0.34**	**6.25**

10) SOS-Chromotest Solid Phase Test

	Puddle mud	**Field soil**
Genotoxicity CIP* value	**17**	**10**
Cytotoxicity CIP value	**4**	**2**

CIP*= colour Index Profile

DISCUSSION

Mitochondria contain multi-component enzyme complexes that conduct vital cellular processes. To produce energy for the cell, this complex of more than 60 enzymes must function in a highly concerted fashion. The mitochondria are therefore vulnerable to a wide variety of toxicants, almost any toxic substance will interfere with some part of the process and evoke a toxic response. Because different classes of toxicants selectively affect different enzymes, several SMP-based protocols have been developed to help differentiate various toxicant groups.

Our laboratory uses two complementary assays, the Forward Electron Transfer (FET SMP) and the Reverse Electron Transfer (RET SMP). In the FET assay the disappearance of NADH is monitored as it is oxidized to NAD^+. In the RET assay, NAD^+ is reduced to NADH. The assays differ in their sensitivity to particular toxicants. For instance RET assay is impacted by chemicals which impact on mitochondrial respiratory enzyme complexes I, II, and V while the FET assay is impacted by chemicals which have an effect on the mitochondrial respiratory enzyme complexes I, III and IV (Blondin et al. 1989).

From the SMP data it can be seen that the June sample contained the greater concentration of toxicants and that there were at least two different toxic bioavailable chemicals/compounds in the sample. The difference between the two samples appears consistent for both bioassays (≈20%). This may indicate that the toxicant load from the road is always being replenished by tire actions, car exhausts, motor drippings, water soluble leachates from the road surface and rain dissolved chemicals. If the above is valid then it would appear that any rain event will produce a toxic/genotoxic runoff.

The nematode bioassay as used in our laboratory provides us with three end-points. Under adverse conditions the animals will respond by arresting their growth or dying. By monitoring a controlled population of 100 J2 animals over their 96 hour growth period, we can measure both lethal and sublethal effects of a tested sample. The number of animals that die, indicates the lethal effect while the number of animals remaining at the J2, J3 and J4 stage provides a measure of the sublethal effects (Samoiloff et al. 1980). Many known mutagens will selectively inhibit the J4 to adult molt, and this inhibition of growth can be used as an indicator of potential mutagenicity in a sample.

In these samples it can be seen that the Aug 3 runoff waters (10X) were more toxic, had the greater sublethal effects and a very much greater mutagenic activity. Even in the unconcentrated samples the August 3rd sample showed the presence of potential mutagenic compounds. These results tend to indicate that long moderate rainfalls continue to create toxic/genotoxic runoffs for the length of the rainfall. However the concentrated samples also indicated that short heavy rainfalls do contribute chemicals to the runoff which have chronic and mutagenic effects on *Panagrellus redivivus.*

The Microtox test, which is based on the inhibition of light production by the bacterium *Vibrio fischeri*, is probably the most internationally used commercial bioassay test. Information on the

toxicity of a sample is obtained through a computer programme which is integrated with the Microtox M500 Toxicity Analyzer. From the data obtained from the runoff samples it can be seen that both 1X samples were negative for toxic activity. However the June 27 10X concentrate showed an EC_{50} effect at 58.8% of the 10X sample concentration and an EC_{10} effect at 13.8% of the 10X sample. The August 3 10X sample was not able to produce an EC_{10} effect.

From the Microtox data it appears that the first minutes of a rainfall at this site produces a runoff which upon concentration is able to produce a toxicity effect in the Microtox test. However the overnight rain appears to have diluted any potential toxicants beyond the sensitivity of the test, even after a 10X concentration.

Spirillum volutans is a large bacterium (≈15 μm wide and ≈40 μm long) which has a bundle of flagella at each end of the cell. The flagellar bundles rotate at about 40 revolutions per second and periodically reverse their direction of rotation. This produces a characteristic pattern of motion which can be disrupted by relatively low levels of certain compounds. The ability of chemicals to effect the synchronized activity of the flagella, led to the development of a toxicity screening test, whose end point is that concentration of sample , chemical or sample dilution which produces a change in 90% (EC_{90}) of the organisms' movements (death or erratic movement) within 120 minutes.

Similar to the SMP observations, in the *S. volutans* bioassay the June 27 sample was more toxic (almost twice) than the August sample. However, it must be noted that the samples required concentration before a typical toxic effect was observed.

Daphnia magna are fresh water crustaceans which are used commonly in Canada to evaluate toxicant presence in water samples. In our tests we use neonates (first instar ≤24h old) whose parents were grown at 20°C with 16/8 h light and dark regimen. All tests using this organism are done in triplicate, (also under the above conditions) with the mean result being reported.

Data from the two runoff samples indicate that the Aug 3rd sample was slightly more toxic to *Daphnia* than the June 27 sample. Also the data suggest that there may be a constant low level replenishing supply of toxicants which may originate from chemicals leaching out of asphalt or are constantly being formed/released by the action of motorized vehicles on the road surface. Notwithstanding the above, it appears that the toxicant load does not necessarily decrease with increased length of rainfall.

The seed germination and root and seedling elongation test using Buttercrunch lettuce seeds provides us with a botanical test which usually is more robust to toxic/genotoxic challenges when compared to microbial based bioassays. In this study, unfortunately only natural runoff and runoff concentrated 10X were evaluated. From the data it can readily be seen that the unconcentrated runoff waters had no effect on seed germination or growth. However when the runoff waters were concentrated 10X none of the seeds germinated. Ideally if more 10X sample had been available the toxicity of the range 1X to 10X would have been evaluated. From these data it may be surmised that over time toxicants will be concentrated in the soils under the downspouts (due to evaporation)

and the natural vegetation will decrease or die off.

Two versions of the SOS-Chromotest were used to screen liquid and solid phase samples for the presence of bioavailable genotoxicants. The SOS-Chromotest is based on the *de novo* synthesis of ß-galactosidase enzyme by a genetically -engineered *E. coli* (strain PQ37). Thus the triggering of the SOS response system in the *E. coli* can be used as a general and early sign of DNA damage. For liquid samples the test is performed in a 96-well microplate and the results are reported as Induction Factors (IF), and IFs between 1.25 -1.3 are considered as the first indication of a positive (genotoxic) result. However a dose response in which the IF is less than 1.25 is considered by some as an indication of potential genotoxicity if the sample were concentrated more.

Although dose response increments in IFs were noted in all four samples (1x and 10x), the Aug. 3 10X sample produced the highest IF value, 1.17. It is surmised that if there were genotoxicants present, their concentration was too low to produce a response in this application of the SOS-Chromotest.

The other version of the SOS-Chromotest used was the direct solid phase SOS-Chromotest (Dutka et al. 1995a). In this version the sample soil and dilutions (or runoff sample) are incubated directly with the *E. coli* cells in antibiotic containing media, following which each of the sample dilutions are transferred to chromogen containing pads for further incubation. The pads are examined for colour production and scored, and these scores are used to develop the CIP (colour index profile) from which a genotoxicity or cytotoxicity value is obtained. Values ≥2 are considered positive and the higher the CIP the more genotoxic or cytotoxic the solid phase sample.

The 1X June 28 runoff was found to have genotoxic properties but no cytotoxins. The cytotoxicity results are similar to the Microyox results. However the solid phase chromogen pad technique which utilizes larger sample volumes and longer contact times than the traditional SOS-Chromotest plate microplate technique appears to be more sensitive for genotoxic effects. The Aug 3 runoff sample was also found to have genotoxic properties but to a lesser degree . Thus this unconventional use of this bioassay tends to confirm that genotoxicants are present in both types of road runoff from this bridge.

The puddle mud was found to contain the greater concentration of genotoxicants and toxicants compared to the grassed field sample. However the differences in toxicant load were not as great as expected. One of the reasons may be that only about 50% of the puddle mud was actually soil particles, thus perhaps diluting the results. The data do suggest that road runoff add to toxicant/genotoxicant load of soils.

The soils, puddle mud and field soil were also tested by the DSTTP for the presence of acute toxicants. This bioassay, based on the Toxi-Chromotest (Kwan 1990) for liquid samples measures the available toxicants of solid phase samples without altering the original characteristics of the sample as occurs in extraction and concentration procedures. Similar to the solid phase SOS-Chromotest the DSTTP is a chromogen pad based test. The results shown in this study indicate that

only 0.34 % of the puddle mud will produce a toxic effect while 6.25 % of the field soil produces a toxic effect. If the nature of the sample is considered, i.e. puddle mud 50 % water and field soil 10 % water the bioassay greatly underestimates the puddle mud toxicity.

While we can be certain of the major source of the toxicants impacting the puddle mud area, the field soil is probably impacted by air borne chemicals as well as rain borne chemicals. Both sites are contaminated the main difference is degree.

SUMMARY

The bioassay data from these two road runoff samples suggest that the two sets of samples trigger responses in different bioassays, thus supporting the need for using the battery of tests approach. The bioassay responses are interesting in themselves as it can be seen that the four laboratory based bioassays (*Panagrellus redivivus, Spirillum volutans, Daphnia magna and Lactuca sativa)* were all more sensitive indicators of toxicity than was the Microtox test, an observation which is very common when testing environmental water samples. The data also indicate that toxicants and genotoxicants are present in all runoffs from this bridge site, i.e. beginning of a rain event and even after 10-15 hours of rain.

The data from this preliminary study using concentrated and unconcentrated runoff water suggest the presence of a very low concentration of acute water soluble toxicants and a greater concentration of water soluble genotoxicants. However the solid SMP results are the most interesting. Since SMP proteins are among the most highly-conserved in nature, only insignificant changes in their structure and function are observable across evolutionary lines. Thus, a quantitative toxic response observed utilizing SMP from beef heart, will likely be exactly duplicated by human, fish or plant SMP, which suggests that highway road runoff may be a serious pollutant.

This preliminary bioassay study of road runoff supports the suspicion that highway runoff could be hazardous to soil organisms and plant life and freshwater aquatic life, not as an acute toxicant but as a chronic and/or genotoxic effect.

These data suggest the need to look at differences in toxicity in soils receiving highway runoffs versus soils not receiving runoff, is there a real difference in toxicant level/effect. Also we believe it is important to establish the role that the various road surfaces play in generating toxic runoff.

REFERENCES

Blondin, G.A., Knobeloch, L.M., Read, H.W. and Harkin, J.M. 1989. An in vitro submitochondrial bioassay for predicting acute toxicity in fish. In Aquatic Toxicology and Environmental Fate: Eleventh Volume, ASTM, STP 1007, G.W. Suter II and M.A. Lewis, Eds. American Society for Testing Materials, Philadelphia.

Borchardt, D. 1993. A framework for the evaluation of ecological impacts of sewer overflow discharges in running waters. In J. Marsalek and H.C. Torno (eds), Proc. 6th Int. Conf. on Urban Storm Drainage, Seapoint Publishing, Victoria, B.C.

Dutka, B.J. 1995. Water and sediment ecotoxicology studies in Mapuche homelands and southern Chile. Report to IDRC, Community-based water quality testing. Contract # 92-1058-02. IDRC, Ottawa.

Dutka, B.J., Bourbonniere, R., McInnis, R., Kwan, K.K. and Jurkovic, A. 1995. Bioassay assessment of impacts of tar sands extraction operations. Environ. Toxicol. and Wat. Qual. 10:107-117.

Dutka, B.J., Teichgräber, K. and Lifshitz, R. 1995a. A modified SOS-Chromotest procedure to test for genotoxicity and cytotoxicity in sediments without extraction. NWRI Contribution NO. 95-53. NWRI, Burlington, Ont.

Dutka, B.J., Marsalek, J., Jurkovic, A., McInnis, R. and Kwan, K.K. 1994. A seasonal ecotoxicological study of stormwater ponds. Zeitschrift für Angewandte Zoologie 80:361-381.

Jones, R.H., Revitt, D.M., Shutes, R.B.E. and Ellis, J.B. 1993. Ecotoxicological impacts of hydrocarbons in an urban aquatic environment. In J. Marsalek and H.C. Torno (eds), Proc. 6th Int. Conf. on Urban Storm Drainage, Seapoint Publishing, Victoria, B.C.

Kwan, K.K. 1990. Direct sediment toxicity testing procedure (DSTTP). NWRI Contribution No. 91-90. National Water Research Institute, Burlington, Ont.

Marsalek J. 1982. Road and bridge-deck drainage design. Research Report RR 228, Ministry of Transportation and communications, Toronto, Ont.

Marselek, J. 1990. PAH transport by urban runoff from an industrial city. In Y. Iwasa and T. Sueishi (eds), Proc. 5th Int. Conf. on Urban Storm Drainage, Osaka, July, 1990.

Mulliss, R., Ellis, J.B., Revitt, D.M. and Shutes, R.B.E. 1993. The ecotoxicological impact of urban discharges upon caged freshwater macroinvertebrates. In J. Marsalek and H.C.

Torno (eds), Proc. 6th Int. Conf. on Urban Storm Drainage, Seapoint Publishing, Victoria, British Columbia.

Samoiloff, M.R., Schulz, S., Jordan, Y., Denich, K. and Arnott E. 1980. A rapid simple long-term toxicity assay for aquatic contaminants using the nematode *Panagrellus redivivus.* Can. J. Fish. Aquat. Sci. 37: 1167-1174.

Versar, Inc. 1985. Management practices for mitigation of highway stormwater runoff pollution, Vol. 4, Executive Summary. Prepared for U.S. Department of Transportation, Federal Highway Administration, Washington, D.C., NTIS PB86-226123.

Woodward-Clyde Consultants. 1989. Pollutant loadings and impacts from highway stormwater runoff. FHWA_RD_88-088, prepared for U.S. Department of Transportation, Federal Highway Administration, Washington, D.C.

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