FINAL REPORT TO TOXFUND ON 1984-1985 ACTIVITIES
NORTH SASKATCHEWAN RIVER TOXICS SURVEY

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OCCURRENCE AND PATHWAYS OF TOXIC CHEMICALS IN THE NORTH SASKATCHEWAN RIVER

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EXECUTIVE SUMMARY

This survey is part of a continuing study of pathways and of ecotoxicological effects of toxic contaminants in the North Saskatchewan River and in Tobin Lake, the principal sink for suspended sediments of the Saskatchewan Basin. This research program, although managed by the National Water Research Institute (NWRI), is a shared program between the NWRI of Inland Waters Directorate and the Environmental Protection Service, Western & Northern Region. This program has broad relevance to toxic chemical management, both in terms of environmental sensing procedures and of control protocols in river and river lake systems in Canada.

The immediate objective is to determine the pathways and significance of priority and unlisted toxic chemicals in water, suspended sediments, and to evaluate toxic effects through use of two different bioassay procedures (Ames and P.redivivus). By contrasting the pathways, chemistry and toxicological response in two different river regimes (high versus low flow), we expect to be able to make significant statements about new and more cost-efficient methods for carrying out toxic chemical sensing in aquatic systems.

The North Saskatchewan River was divided into nine study sites of approximately equal distance, from Devon (above Edmonton) to Nipawin, almost 1000 km downstream. Sampling was carried out during low summer flow in 1983 when one should expect to see point sources dominating the river chemistry. Sampling was carried out again in spring of 1984 when one should find significant contributions from nonpoint sources. Whole water, and suspended sediments were collected from each site. Bottom sediments were collected where sediments were sand-sized and smaller. Each site has been characterized for a suite of physical and chemical variables. Data reported here refer mainly to the 1983 low flow data set for which the analytical results for priority chemicals, metals, and bioassays are now complete.

The chemistry of metals is typical of prairie rivers with high concentrations in whole water samples during high water and low concentrations at reduced level of flow. Suspended sediment is enriched immediately below the metropolitan area of Edmonton, however concentrations decline downstream to levels commensurate with background, possibly due to dilution by unenriched sediment inputs into the system. Bottom sediments are impoverished in metals relative to suspended sediments and cannot be

used to indicate downstream trends either for water or suspended sediment metal chemistry.

Organic chemistry, combined with two independent bioassays - the P.redivivus and Ames tests, lead to critical questioning of the value of current priority chemical screening practices for environmental sensing purposes. Our observations are especially germane as the data should reflect the influences of point sources.

Although pesticides were detected in all water samples at trace levels (1-5 ppt), priority chemicals were detected at only one site; this extract was not toxic. Toxicity was observed for only one water sample but no priority chemicals were detected.

The most toxic responses were observed for the acid fractions in the suspended sediments. Seven of the nine sites sampled showed toxicity in both bioassays. Priority phenols were found in three of the seven toxic fractions; no priority chemicals were detected in the remaining four toxic fractions.

There is little comparability between toxic response of whole water samples and suspended sediments contained in the whole water samples.

No conclusive toxicity was observed in the bottom sediment samples.

The following questions arise, therefore:

1. Toxic response in the absence of Priority Chemicals implies that there may be profound deficiencies with the menu-driven approach to environmental sensing of toxic chemicals.

2. The much greater toxic response and larger number of priority chemicals associated with suspended sediments implies that water may be an inadequate medium upon which to base toxic chemical criteria for toxic chemical sensing purposes in aquatic systems.

3. Our data suggest that certain priority chemicals which exist in water or on sediments, produce no toxic response in one or both of the bioassay procedures.

4. Routine chemical screening of water samples for organic compounds is expensive, generally produces ND values, misses not only many toxic compounds which are associated with suspended sediment but also those compounds which are not part of the screening menu, and can provide no ecologically relevant information for those compounds.

Full reporting of the results of this study is scheduled for FY85-86. We make no value judgements concerning the quality of water in the North Saskatchewan River on the basis of the data presented here.

PRODUITS CHIMIQUES TOXIQUES DE LA RIVIÈRE SASKATCHEWAN-NORD: VOIES D'ENTREE

RAPPORT FINAL SOUMIS AU COMITÉ DU TOXFUND (FONDS POUR LES CONTRATS DE RECHERCHE SUR LES PRODUITS CHIMIQUES TOXIQUES) SUR LES ACTIVITÉS 1984-1985

ÉTUDE SUR LES TOXIQUES DE LA RIVIÈRE SASKATCHEWAN-NORD

RÉSUMÉ ADMINISTRATIF

Cette étude s'inscrit dans le cadre de travaux permanents sur les voies d'entrée et les effets écotoxicologiques des toxiques qui polluent la rivière Saskatchewan-Nord et le lac Tobin, le principal réservoir de sédiments en suspension du bassin de la Saskatchewan. Ce programme de recherche, quoique dirigé par l'Institut national de recherche sur les eaux (INRE), est un travail de collaboration entre l'INRE de la Direction générale des eaux intérieures et le Service de la protection de l'environnement, Région ouest et nord. Il s'applique dans une large mesure à la gestion des produits chimiques toxiques, tant au point de vue des méthodes de détection dans l'environnement qu'au point de vue des mesures de dépollution des bassins hydrographiques du Canada.

Le premier objectif de ce programme est de déterminer les voies d'entrée et l'importance des produits chimiques toxiques d'intérêt prioritaire et non répertoriés, présents dans l'eau et les sédiments en suspension, et d'évaluer leurs effets toxiques au moyen de deux épreuves biologiques différentes (Ames et P. redivivus). En comparant les voies d'entrée des produits chimiques présents et les réactions de toxicité au cours de deux régimes différents (débit de crue par rapport au débit d'étiage), nous prévoyons qu'il sera possible de tirer des conclusions significatives sur de nouvelles méthodes plus rentables permettant de déceler des produits chimiques toxiques en milieu aquatique.

La rivière Saskatchewan-Nord a été divisée en neuf sites d'étude situés à des distances pratiquement égales, allant de Devon (au-dessus d'Edmonton) à Nipawin, soit environ 1000 km en aval.

On a effectué un échantillonnage durant l'été 1983, période d'étiage où les produits chimiques décelés devaient provenir en très grande partie de sources ponctuelles. On a réalisé un nouvel échantillonnage au printemps 1984, période où les sources diffuses devraient contribuer de façon importante à l'apport de produits chimiques toxiques. On a recueilli dans chaque site des échantillons d'eau entière et de sédiments en suspension. Des sédiments de fond ont été prélevés aux endroits où la grosseur de leurs particules était inférieure ou égale à celle des grains de sable. On a établi pour chaque site une série de variables physiques et chimiques. Les données signalées ici portent essentiellement sur la série de données obtenues en 1983 avec en débit d'étiage et pour laquelle on dispose maintenant de tous les résultats analytiques concernant les produits chimiques d'intérêt prioritaire, les métaux et les épreuves biologiques.

La concentration des métaux est caractéristique des cours d'eau des Prairies: elle est élevée dans les échantillons d'eau entière pendant le débit de crue et faible pendant le débit d'étiage. La concentration de métaux dans les sédiments en suspension est plus élevée juste au-dessous de la région métropolitaine d'Edmonton, mais elle diminue en aval pour atteindre une valeur comparable a la concentration de base, vraisemblablement parce qu'il se produit une dilution due à l'apport de sédiments dont la teneur en métaux ne s'est pas accrue. Les sédiments de fond sont pauvres en métaux par rapport aux sédiments en suspension et ne peuvent servir à indiquer quelle tendance suit en aval la teneur en métaux de l'eau ou des sédiments en suspension.

Si l'on examine la teneur en composés organiques et les résultats de deux épreuves biologiques différentes (épreuve avec P. redivivus et test d'Ames), on est porté à remettre en question le mérite des méthodes utilisées actuellement pour déceler des produits chimiques d'intérêt proiritaire dans l'environnement. Nos observations sont particulièrement pertinentes car les données devraient refléter l'influence des sources ponctuelles.

Même si l'on a décelé des traces de pesticides (1-5 parties par trillion) dans tous les échantillons d'eau, un site seulement renfermait des produits chimiques d'intérêt prioritaire; l'extrait prélevé n'était pas toxique. Un seul échantillon d'eau s'est révélé toxique mais aucun produit chimique d'interêt prioritaire n'a été trouvé.

Les fractions acides des sédiments en suspension ont donné les réactions les plus toxiques. D'après les résultats des épreuves biologiques, sept des neuf sites avaient des échantillons toxiques et l'on a trouvé des phénols d'intérêt prioritaire dans trois des sept fractions toxiques; aucun produit chimique d'intérêt prioritaire n'a été décelé dans les quatre autres fractions toxiques.

Il n'est guère possible de comparer la toxicité des échantillons d'eau entière à celle des sédiments en suspension contenus dans des échantillons d'eau entière.

Les observations n'ont pas permis de tirer des conclusions quant à la toxicité des échantillons de sédiments de fond .

Par conséquent, on est amené à se poser les questions suivantes:

1. D'après la toxicité observée en ne tenant pas compte des produits chimiques d'intérêt prioritaire, l'approche qui se limiterait à certains produits pour déceler des toxiques chimiques dans l'environnement présenterait de graves lacunes.

2. On observe une toxicité beaucoup plus importante et un nombre de produits chimiques d'intérêt prioritaire associés aux sédiments en suspension plus élevé, c'est pourquoi l'eau ne constitue peut-être pas un milieu approprié sur lequel baser des critères relatifs à la détection de produits chimiques toxiques dans les systèmes aquatiques.

3. Nos données indiquent que certains produits chimiques d'intérêt prioritaire, présents dans l'eau ou sur des sédiments, ne donneraient aucune réaction toxique avec l'une ou l'autre des épreuves biologiques.

4. Il est coûteux d'analyser régulièrement des échantillons d'eau pour y déceler des composés organiques et on n'obtient généralement pas de valeurs déterminées; non seulement un bon nombre de toxiques associés aux sédiments en suspension mais les composés exclus de la liste des produits à déceler passent inaperçus et les renseignements obtenus sur les composés ne présentent pas d'intérêt écologique.

Un rapport complet sur les résultats de l'étude devrait paraître pour l'année financière 85-86. Nous ne nous prononcerons pas sur la qualité de l'eau de la rivière Saskatchewan-Nord d'après les données présentées ici.

OCCURRENCE AND PATHWAYS OF TOXIC CHEMICALS

IN THE NORTH SASKATCHEWAN RIVER

FINAL REPORT TO TOXFUND ON 1984-85 ACTIVITIES
"NORTH SASKATCHEWAN RIVER TOXICS SURVEY"

BACKGROUND

This survey is part of a continuing study of pathways and of ecotoxicological effects of toxic contaminants in the North Saskatchewan River and in Tobin Lake, the principal sink for suspended sediments of the Saskatchewan Basin. (Figure 1). This research program is a horizontally integrated activity in which research is seen to be directly in support of operational objectives both of the Inland Waters Directorate and of the Environmental Protection Service in Western and Northern Region. Both agencies have contributed substantial A-Base support to the program over a number of years. Since 1982, program management has been exercised by the National Water Research Institute on behalf of both agencies. We believe that this program has broad relevance to toxic chemical management, both in terms of environmental sensing procedures and of control protocols in river and river-lake systems in Canada.

OUTPUTS FOR FY84-85

Because major reporting activities are scheduled for FY85-86, this report will focus on objectives, methods, and preliminary data and analysis completed for 1983 water and sediment samples. We include some limited geochemical data for 1984 samples. The outputs identified for TOXFUND are:

1. Analysis of North Saskatchewan River samples for organochlorines and priority pollutants.

2. Performance of ecological testing on toxic fractions.

3. Data interpretation and report preparation comparing spring high-flow data with summer low-flow data.

STUDY OBJECTIVES 1983-1986

The outputs for 1984-85 should be seen within the larger context and overall objectives of this study. These are:

1. To determine the presence, pathways and significance of priority and unlisted toxic substances present in water and in suspended and bottom

sediments of the North Saskatchewan River and in bottom sediments of Tobin Lake.

2. To develop *in situe* biological techniques and bioassay procedures for detection and assessment of toxic substances. This includes surveying the responses of benthic communities and testing for lethality, mutagenicity, and developmental inhibition by nematode (*P. redivivus*) (Samoiloff *et al*, 1983) and bacterial bioassay (Ames) analyses.

3. To relate the concentrations of toxic substances found, to morphological abnormalities in the biological community, and the physicochemical characteristics of the sediments in Tobin Lake.

4. To evaluate the biogeochemical processes which determine the characteristics and degradation products of selected organic contaminants during transport within a selected reach of the North Saskatchewan River.

5. To determine the nature of ecological impact of selected organic contaminants on benthic organisms within a selected reach of the North Saskatchewan River.

6. To determine the relative contributions of point and diffuse sources to mass flux of contaminant transport in the North Saskatchewan River, and the role of hydrologic regime in contaminant transport over the length of the North Saskatchewan River.

7. To evaluate implications for environmental sensing and management of toxic chemicals in the North Saskatchewan River in particular, and in Canadian Rivers in general.

FIELD PROGRAM

The field program was designed to contrast the chemistry of water and sediments of high discharge versus low discharge conditions. Studies of prairie rivers (Blachford & Ongley, 1984) and elsewhere (Ongley *et al.* 1981) have indicated that nutrient and metal chemistry of rivers during high flow tend to reflect the influence of diffuse (nonpoint) sources within the drainage basin; alternatively, low flow is mainly influenced by major point sources upstream. The objective of the field program was, therefore, to establish whether regime sampling of riverine organic chemistry will permit effective discrimination of point versus diffuse source impacts for toxic organic chemicals.

It is well-known that many of the priority pollutants are preferentially associated with sediment and biota (Chapman *et al*, 1982). The 1983 and 1984 field program focussed upon whole water, suspended sediment, and bottom sediment. A parallel study of biological effects is underway. The objective was to establish the substrate in which priority pollutants are preferentially transported in this river system. To our knowledge, this type of program has never before been attempted. If successful, it will add immeasurably to our knowledge of contaminant pathways in prairie river systems.

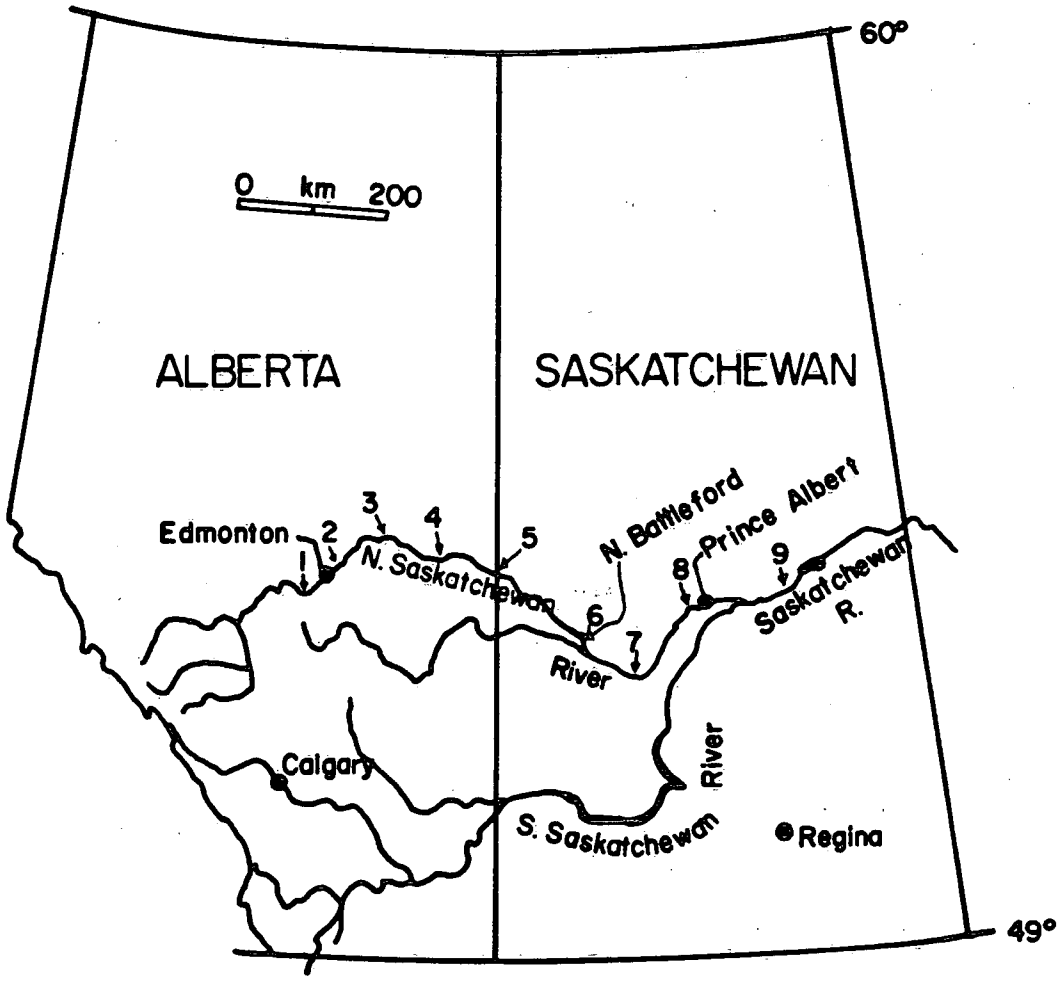

FIGURE 1: Sampling sites on the North Saskatchewan River including the Saskatchewan River near Nipawin (site #9).

The river was divided into nine reaches (Figure 1). The selection of sites reflect existing data bases of federal and provincial agencies, plus an earlier study of storm-flow chemistry carried out in 1981. The sites were selected to reflect lateral mixing across the section. With the exception of the Fort Saskatchewan site, sites were selected so that each would be many kilometres downstream from known point sources or from inflowing tributary rivers. The objective was not to measure impact of particular point sources immediately upstream, but rather to assess the collective impact of point and diffuse sources located a considerable distance upstream.

The river sections used in this study are:

1. Devon: background site located upstream of the city of Edmonton.

2. Fort Saskatchewan: immediately upstream of a major petrochemical complex. The data mainly represent the effect of Edmonton. The river is not laterally mixed at this site.

3. Pakan: at site of a former ferry crossing. Alberta Environment has concluded that the North Saskatchewan River is sufficiently mixed at this point for use as their principal monitoring site below the Edmonton-Fort Saskatchewan complex.

4. Myrnam Bridge: At Alberta Highway 881.

5. Highway 17: upstream of the ferry crossing and the new bridge under construction at the Saskatchewan/Alberta border. The site is near the water intake for the city of Lloydminster.

6. Battlefords: immediately upstream of Highway 4. This is upstream of all point sources in this urban area and upstream of the Battle River confluence.

7. Borden Bridge: immediately upstream of the bridge on Highway 16, northwest of the city of Saskatoon.

8. Prince Albert: several kilometres upstream of Prince Albert at the site of the former Crutwell ferry.

9. Nipawin: The site is in the reach upstream of the Nipawin power dam development. The site is not to be flooded until 1985; there was no disturbance during the study period. The site is well downstream of the confluence of the North and South Saskatchewan Rivers.

Sampling Program: Water

With one exception, water sampling was carried out at mid-stream (zone of maximum flow). At Fort Saskatchewan where the river is not laterally mixed, sampling for organic chemistry in water was distributed between two sampling points, one at one-third and a second at the two-thirds position

in the cross section. Samples for other water variables at Fort Saskatchewan were from mid-stream. Whole and filtered water samples for physical characteristics and for nutrient and inorganic chemistry and were collected from a depth of 1/3m in mid-stream, in replicate. In situ measurements of conductivity, pH, turbidity and temperature were made during each collection period per site.

Water for total and filtered metals was sampled into 1L and 250 mL polyethylene bottles respectively. The bottles were precleaned and soaked in 1:1 distilled water - concentrated HNO_3 for approximately one week, then rinsed in distilled water at least five times. Sample was preserved in the field by acidifying with reagent grade HNO_3 to a final concentration of 0.5% of nitric acid. Filtration was carried out either immediately on site or within a few hours of sampling, depending upon weather and length of program at each site. Filtration was through acid-rinsed glass fiber filters using a GeoFilter assembly.

Whole water for total mercury analysis was sampled directly into 250 mL Pyrex bottles which had been muffled at 450°C for approximately six hours; the ground-glass stoppers were cleaned in 1:1 distilled water - HNO_3 then washed in distilled water and oven-dried. After cooling, 3 mL concentrated HNO_3 and 1 mL of 5% $K_2Cr_2O_7$ was added to the prepared bottle for subsequent preservation of sample. The assembled bottle and stopper are protected at all times by a whirl-pak bag attached over the bottle top. Spare, pretreated mercury bottles were carried throughout the field program, then analyzed as blanks as part of the quality assurance program. Methyl mercury was determined from whole water collected into either 2 x 4L or 1 x 8L polyethylene bottle which had been precleaned. The sample was preserved with 15 mL concentrated H_2SO_4 per 5L of water.

Water for total and total dissolved phosphorus was sampled directly into precleaned 50 mL Sovirel bottles supplied by the Saskatoon Laboratory of the Water Quality Branch of Inland Waters Directorate. The filtered sample was taken during filtration for metals.

Distilled water carried in the field was sampled periodically, as for heavy metals, and subsequently analyzed as blanks. All samples for routine water chemistry noted above were kept under refigeration in the field. Because of distances involved, samples for phosphorus (not reported here) were shipped to the Water Quality Branch Laboratory weekly.

Whole water samples for organic chemical analysis were collected into 4L, amber glass jugs. These jugs had previously contained distilled in glass solvents (such as dichloromethane) and were used without additional preparation. The samples were collected in the same manner as described above for other water chemistry. The jugs were submersed off the forward bow of the research vessel to a depth of approximately 20 cm during filling Each jug was preserved in the field by the addition of 100 mL of dichloromethane (DCM) and shaken end-over-end for at least one minute. These jugs were shipped to the EPS organic laboratory where they were held at +2.0°C until analysis. For the purpose of quality assurance and to enhance analytical sensitivity, either two or four 4L jugs were collected (8 to 16 L). Because of the length of time required for time-integrated sediment sampling, 4L samples were taken at approximately equal intervals

over the sampling period. Sample volume per site is noted in Table 1 below:

TABLE 1: Field Sampling — Volumes and Duration

	Summer-1983			Spring-1984		
	Organic Water Sample	Sampling Duration Susp. Sed.	Q*	Organic Water Sample	Sampling Duration Susp. Sed.	Q*
1. Devon	4 x 4L	7 h.	136	4 x 4L	2. h.	293
2. Fort Saskatchewan	2 x 4L	7 h.		2 x 4L	1.5 h.	
3. Pakan	4 x 4L	7 h.		4 x 4L	1.5 h.	
4. Myrnam Bridge	2 x 4L	7 h.		2 x 4L	1.5 h.	
5. Highway 17	2 x 4L	7 h.	132	2 x 4L	1.5 h.	326
6. Battlefords	4 x 4L	6 h.		4 x 4L	1.5 h.	
7. Borden Bridge	2 x 4L	5 h.		2 x 4L	1.5 h.	
8. Prince Albert	4 x 4L	4 h.	175	4 x 4L	3. h.	351
9. Nipawin	4 x 4L	4 h.		4 x 4L	2. h.	

* Daily mean discharge in $m^3 s^{-1}$

Sampling Program: Suspended Sediment

Suspended sediment was collected from a depth of approximately 0.3 m using the Envirodata Sedisamp System-II continuous-flow centrifugation apparatus. This apparatus, described in Ongley and Blachford (1982), quantitatively extracts suspended sediment from the water column to produce a time-integrated bulk sample. Sampling time (Table 1) depends upon amount of sample required and concentration of suspended sediment in that sampled medium. Little Giant #1-42 and 2E series submersible pumps pumped river water through precleaned rubber tubing directly into the continuous-flow apparatus using a polypropylene ball valve at a rate of $4 L.min.^{-1}$. The hoses were precleaned by pumping a 2% solution of Extran 300 in tapwater through a coil of hose for three hours, then rinsed with tapwater for 0.5 h followed by circulation of a 5% solution of reagent grade HNO_3 through the coil for a further 1.0 h., and finally by distilled water for another 1.0 h. The coil was carried into the field and 2.5 m lengths per pump cut off as required at each site. River water was pumped through the hose and control valve for 10 minutes prior to sampling.

Recovery of suspended sediment (mineral + organic matter) was generally better than 95% for the size range 62-0.2um.. Pump intake nozzles were protected from large organic fibers (floating weed) by a protective aluminum screen. All rubber tubing was discarded after each site to avoid carry-over contamination. Independent tests of potential contamination by priority chemicals which might originate within the Sedisamp and peripheral apparatus, were made by the Organics Laboratory of the Environmental Protection Service, Edmonton. No evidence for internally generated contamination was detected (D.Birkholz, draft report) There is also no

evidence of internal contamination in field samples, such as constant appearance of one or more priority chemicals, in either this or other similar programs.

One Sedisamp was used to collect sediment for inorganic and physical analyses of suspended sediment; a second Sedisamp was used exclusively for sediment destined for organic analysis. Centrifuge bowls were precleaned, then rinsed in distilled-in-glass grade acetone prior to use. Sediment used for inorganic and physical analyses was cleaned from the bowl into a plastic bag and immediately frozen; these samples were later freeze-dried before chemical and physical analysis. Sediment used for organic analysis was cleaned out of the centrifuge bowl with an acetone-rinsed stainless steel spatula into acetone-rinsed aluminum foil; the sample was double wrapped in foil, then immediately frozen for storage pending organic (priority pollutant) analysis.

All suspended sediment sampling was carried out at mid-stream, as noted above, with the single exception of the Fort Saskatchewan site. Both centrifuge systems were operated in a 4.6m vessel using a Pincor 4KW generator. Generator exhaust was routed overboard to below waterline to avoid contamination from exhaust fumes.

Sampling Program: Bottom Sediments

The North Saskatchewan River is a cobble and gravel-bed river for much of its upper course. This gives way to a mobil sand bottom in the vicinity of Myrnam Bridge. Gravel appears again at the Nipawin site. Bottom samples were taken, where possible, with a Ponar dredge. Generally, samples were possible at:

Myrnam Bridge
Highway 17
Battlefords
Borden Bridge
Prince Albert

In 1983, bottom samples were taken at the one-third and two-thirds position across the section at each sampled site. In 1984, a single bottom sample was taken from mid-stream. Sample material for physical and inorganic measurements was bagged in plastic bags and held in a cool environment, then freeze-dried. Sample for organic analysis was double wrapped in acetone-rinsed aluminum foil, placed in glass jars with teflon-lined lids, then frozen.

Field Program: Benthic Biology

Species composition, abundance, and chemical burden is an indicator of health of the aquatic environment. A survey of benthic invertebrates was undertaken at each of the nine sites in summmer of 1984. A summary of organisms collected at each site is provided in Table 2. Analysis is scheduled in 1985.

TABLE 2

SUMMARY OF ORGANISMS COLLECTED AT EACH SASKATCHEWAN RIVER SITE

ORGANISM	DEVON	FORT SASK.	PAKAN	MYRNHAM	HWY. 17	BATTLE-FORDS	BORDEN	LA COLLE	WELDON
Oligochaeta, large			X						
Oligochaeta, small				X	X				
Helobdella stagnalis		X					X		X
Glossiphonia complanata		X	X		X	X	X		X
Dina dubia			X	X					
Erpobdella punctata		X		X	X	X	X		
Nephelopsis obscura					X				
Sphaeriidae			X	X	X	X	X	X	X
Anodonta sp.		X					X		X
Lasmigona complanata									X
Gastropoda		X	X	X	X	X	X	X	X
Amphipoda									X
Decapoda									X
Heptagenia sp.	X	X	X	X	X	X	X	X	
Stenonema sp.									X
Hexagenia sp.									X
Anisoptera				X	X	X	X	X	X
Ichnura sp.									X
Acroneuria sp.	X				X	X	X		
Pteronarcys sp.						X			
Hydropsychidae		X	X	X	X	X	X	X	X
Brachycentrus sp.								X	
Limnephilidae								X	X
Polycentropus sp.									X
Neuroptera						X	X		
Tipula sp.			X						
Chironomidae				X				X	

ANALYTICAL PROGRAM

Physical Characteristics

Particle-size characteristics of suspended sediments were determined following methods of Duncan & LaHaie (1979), Vitturi & Rabitti (1980), and KcKeague (1976) on a 0.5 to 1.0g subsample of freeze-dried sediment. Organic matter was oxydized using a 30% solution of hydrogen peroxide. The sample was then dispersed in a 0.5% solution of sodium hexametaphosphate in a Bransonic ultrasonic bath for five minutes, then run on a Micromeritics Sedigraph 5000 or 5000ET. The bottom sediments are predominantly sand or larger particles. This freeze-dried material was homogenized and a 200 g sub-sample placed in the top of a sieve stack consisting of integer phi sizes from -1.0 to 4.0. The sample was sieved in a rotary sieve shaker for 10 minutes after which the material captured in each sieve was weighed. The <4 phi (<62.5 um) fraction was always less than 0.5% of the original sample and was not, therefore, recorded.

phi	-1	=	2000	um
phi	0	=	1000	um
phi	1	=	500	um
phi	2	=	250	um
phi	3	=	125	um
phi	4	=	62.5	um

Heavy Metals: Water

Fe, Mn, Pb, Cu, Ni, Cd, Zn and Cr in filtered and unfiltered waters were determined by graphite furnace atomic absorption spectrophotometry (AAS) without digestion. As and Se in filtered and unfiltered waters were determined using semi-automated flameless boro-hydride reduction with AAS. Total mercury was analyzed by wet digestion, reduction with stannous sulphate, trapping on a silver plug, and subsequent heat-pulsing into an absorption cell and read by AAS. Methyl mercury in unfiltered water was determined by organic extraction, concentration, and injection into a gas chromatograph using an electron capture detector (ECD).

Heavy Metals: Sediment

All sediment samples were analyzed in duplicate. Fe, Mn, Pb, Cu, Ni, Zn and Cr were determined in 1.0g of freeze-dried sediments after digestion with nitric-perchloric acid and volumed to 25.0 mL, using AAS. As and Se were digested as above using 0.25g samples, volumed to 25.0 mL having a final HCl concentration of 30.0%, and read using semi-automated flameless borohydride reduction techique with AAS. Total mercury was determined on a 0.5g sample which was digested with 10.0 mL aqua regia, volumed to 25.0 mL, and read by semi-automated flameless AAS.

Priority Chemicals: Water

Each 4L sample was extracted under neutral pH by the addition of an additional 100 mL DCM asing a 3" teflon stirring bar and magnetic stirrer. Extraction was carried out for 30 minutes after which the DCM was removed by pipet and dried by passing through a 50g sodium sulphate column. The dried extract was collected in a 300 mL flat-bottomed flask. Extraction was repeated once more for an additional 30 minutes. The extracts were combined in the 300 mL flask for analysis of **NEUTRAL COMPOUNDS.**

Next, each 4L water sample was acidified to pH 2.0 by the addition of 6N sulfuric acid. The samples were extracted as for Neutral Compounds except that a 2.0 hour period was used. This extract contains **ACID COMPOUNDS.**

The Neutral and Acid extracts were concentrated using a Kuderna-Danish (K-D) apparatus. Since more than one 4L jug was obtained at each site, the extracts for each of the Neutral and Acid components were combined and concentrated to 5 mL using a combination K-D and nitrogen blowdown methods.

A micro silica-gel cleanup technique was used for the clean-up of water extracts prior to determination of pesticides. The cleaned-up extracts were transferred to labelled vials and stored as -40^{o}C until GC/ECD analysis.

Priority Chemicals: Sediments

The amount of sediment available for extraction was variable. Generally, limitations imposed by very low suspended concentrations during period of low flow (e.g. summer, 1983) restricted sample size. The following procedure describes how organic contamintants are extracted from sediments into five fractions.

1983 sediment samples of 21-52g were Soxhlet extracted for 16h with 1:1 acetone - hexane. The extract was preconcentrated to ca. 50 mL usng a rotary evaporator and tranferred to a 1L separatory funnel containing 250 mL of HPLC grade water. 6N sodium hydroxide was added and the mixture extracted with 120 and 60 mL of DCM. After extraction, the DCM was removed, dried by passage through a 50g sodium sulphate column and collected in a 300 mL flat-bottomed flask. This extract contains the **BASE/NEUTRAL (B/N) COMPOUNDS.**

The B/N extracts were preconcentrated to ca. 5 mL using a K-D apparatus, then further preconcentrated to ca. 1 mL using nitrogen blowdown. The DCM was exchanged into hexane by the addition of 5.0 mL hexane followed by mixing and preconcentration to ca. 2 mL using nitrogen blowdown. This extract was contained in a Mills tube and stored at $+2^{o}$C for chromatography.

The residual aqueous phase from the initial B/N extraction was then adjusted to pH <2.0 using 6N sulphuric acid and extracted with 120 and 60 mL of DCM. The solvent was recovered as above. This extract contains the **ACID COMPOUNDS.**

The Acid extract was preconcentrated to ca. 5 mL using a K-D apparatus and the volume adjusted to 5.0 mL by the addition of DCM or with the aid of nitrogen blowdown. These Acid extracts were stored in 7.0 mL amber vials at -40°C until solvent exchange.

Sediment cleanup for B/N Compounds employs the method of Birkholz et al. 1983. The entire B/N extract (in hexane) was applied to a 10 g silica-gel column (fully activated at 130°C). The column was then eluted with 80 mL hexane and the **FRACTION 1 COMPOUNDS** collected in a 125 mL flat-bottomed flask.

The Mills tube containing B/N compounds was then rinsed with 3 x 5 mL of 20% DCM in hexane to dissolve any hexane insoluble matieral. These rinsings were transferred to the chromatographic column and the applied material eluted with 85 mL of 20% DCM in hexane. The eluant was collected in a 150 mL flat-bottomed flask as **FRACTION 2.**

The Mills tube was then further rinsed with 3 x 5 mL of 60% DCM in hexane and the rinsings transferred as above. The applied material was then eluted with 85 mL of 60% DCM in hexane; the eluant was collected as before as **FRACTION 3.**

Final elution of the column was with 50 mL DCM followed by 50 mL methanol. The eluant was collected as before as **FRACTION 4.**

Each successive fraction was preconcentrated to ca. 4 mL using a K-D apparatus and then bulked up to 5.0 mL using DCM.

Prior to biological assay by P. redivivus, an aliquot (2.0 mL) was removed and saved for chemical analysis. The remaining 3.0 mL was exchanged into dimethyl sulfoxide (DMSO). This involved preconcentration of the extract to ca. 1 mL using nitrogen, 2 mL of DMSO was added and the mixture preconcentrated to 2 mL using nitrogen. The extact was then reconstituted to 3.0 mL using DMSO. After this exchange, the extracts were transferred to amber vials with teflon-lined screwcaps such that 1.0 mL was available for P.redivivus bioassay, and 2.0 mL was available for Ames bioassay.

Priority Chemicals: Instrument Analysis

Analysis for **pesticides** was conducted using a Varian model 6000 chromatograph equiped with a capillary injection system, two Ni^{63} electron capture detectors (ECD), autosampler, and a VISTA model 402 data handling system. Pesticide screening was performed using two 30m x 0.25mm fused silica capillary columns, one coated with DB-1 and the other with DB-5. Conditions for analaysis were as follows:

Temperatures (°C): inlet, 270; column, 70 for 2 min., 6/min. to 300, 5 min. hold; detector, 300.

Carrier gas: linear velocity 20 $cm.sec^{-1}$.
Makeup gas: Ar/CH_4 (95:5) at 30 $mL.min^{-1}$.

Injection mode: splitless, purge time 1.0 min.
Injection volume: 2uL
Chart Speed: 1 $cm.min^{-1}$

Analysis for **priority chemicals** was conducted with a Hewlett-Packard model 5993 GC/MS/DS. Using a 30m x 0.25mm ID fused silica column coated with DB-5, the conditions were:

Temperatures (^{o}C): inlet, 270; column, 40 for 4 min., 10/min. to 300, 10 min hold; ion source, 250.

Carrier gas: He, linear velocity, 20 $cm.sec^{-1}$.
Injection mode: splitless, purge time, 30 sec.; injection volume, 3uL
Ionization mode, electron impact (70eV); masses scanned, 50 to 500.

Priority Chemicals: Quality Control/Quality Assurance

1. Water: 4L samples of North Saskatchewan River water were obtained from the E.L. Smith Water Treatment plant and by the National Water Research Institute at the Devon sampling site. These samples were fortified with pesticides, phenols and B/N priority pollutants.

2. Sediments: 50g aliquots of wet bottom sediment obtained from Prince Albert, Saskatchewan were fortified with pesticides, phenols and B/N priority pollutants. This is discussed below.

3. Analytical error: method blanks were generated and analyzed as a check for artifacts which may be introduced during sample workup.

Biological Testing

Extracts and/or fractions which had been exchanged into DMSO were assayed using two tests.

1. Ames: the Salmonella typhimirium microsome mutagenicity test employed the following test strains: TA97, TA98, TA100, and TA102. Tests were conducted with and without liver activation (ie. addition of S9). Details of the test are described elsewhere (Birkholz, 1982; Samoiloff et al, 1983).

2. P.redivivus: This bioassay employs the free-living nematode P.redivivus. Response to the extracts are recorded relative to the response observed for a control extract and the results ranked. Details are presented elsewhere (Samoiloff et al, 1980; Samoiloff et al, 1983; and Samoiloff and Bogaert, 1984).

Quality control/assurance was maintained by submitting a B/N and Acid extract obtained from a water sample which had been fortified with pesticides and phenols.

RESULTS

Full reporting of the results of this study is scheduled in FY85/86. We present here a selection of results which have been completed to date. It is important to note that we make NO value judgements concerning the quality of water on the basis of these data.

Physical, and other descriptive characteristics

Discharge of the North Saskatchewan River is measured by Inland Waters Directorate at Edmonton, near Highway #17, and at Prince Albert. Sampling was carried out sequentially downstream at times of relatively stable flow. Discharge at these three sites are characteristic, therefore, of intermediate ungauged sites. Discharge on sampled dates is noted on Table 1.

Downstream trends for conductivity, pH, dissolved oxygen, and turbidity are illustrated in Figure 2. Differences between summer low flow (1983) and spring high flow (1984) are obvious. **Conductivity** is lower during high flow at a time when much of the discharge originates from overland flow rather than from groundwater discharge into the channel. The city of Edmonton has a small effect on conductivity during low flows, however the general downstream trend in both seasons is one of increasing conductivity. River **pH** is one pH unit higher during low flow, reflecting both the larger contribution of bases in groundwater and the uptake of CO_2 by phytoplankton during the summer. The latter is especially evident immediately downstream from Edmonton where an algal bloom was evident during the 1983 summer sampling period.

Dissolved oxygen was measured in mid-day and generally exceeds 9 $mg.L^{-1}$, with a large increase associated with primary productivity immediately downstream from Edmonton during the summer. **Turbidity** is very low during the low flow period, indicating lack of resuspension of bottom sediments and small inputs of suspended matter from inflowing tributaries. The general increase in the downstream direction during summer may reflect some combination of increased loading of anthropogenic sediments, and an increasing drainage area associated with agricultural soils and commensurately greater unit discharges of suspended sediment to the North Saskatchewan River by tributaries. As expected, turbidity is much higher during high flow; nevertheless, the declining downstream trend suggests that the sampling program may not have corresponded exactly with rate of downstream transport of suspended sediment. Other sedimentary or dilution processes may also account for the downstream trend.

Sediment particle-size characteristics are illustrated in Figure 3. Suspended sediments, which were sampled close to the surface of the water column, are approximately half silt, half clay, with virtually no sand (>62um) component. Suspended sediments of summer versus spring are only different in terms of organic carbon. Bottom sediment are all sand with less than 0.5% silt/clay.

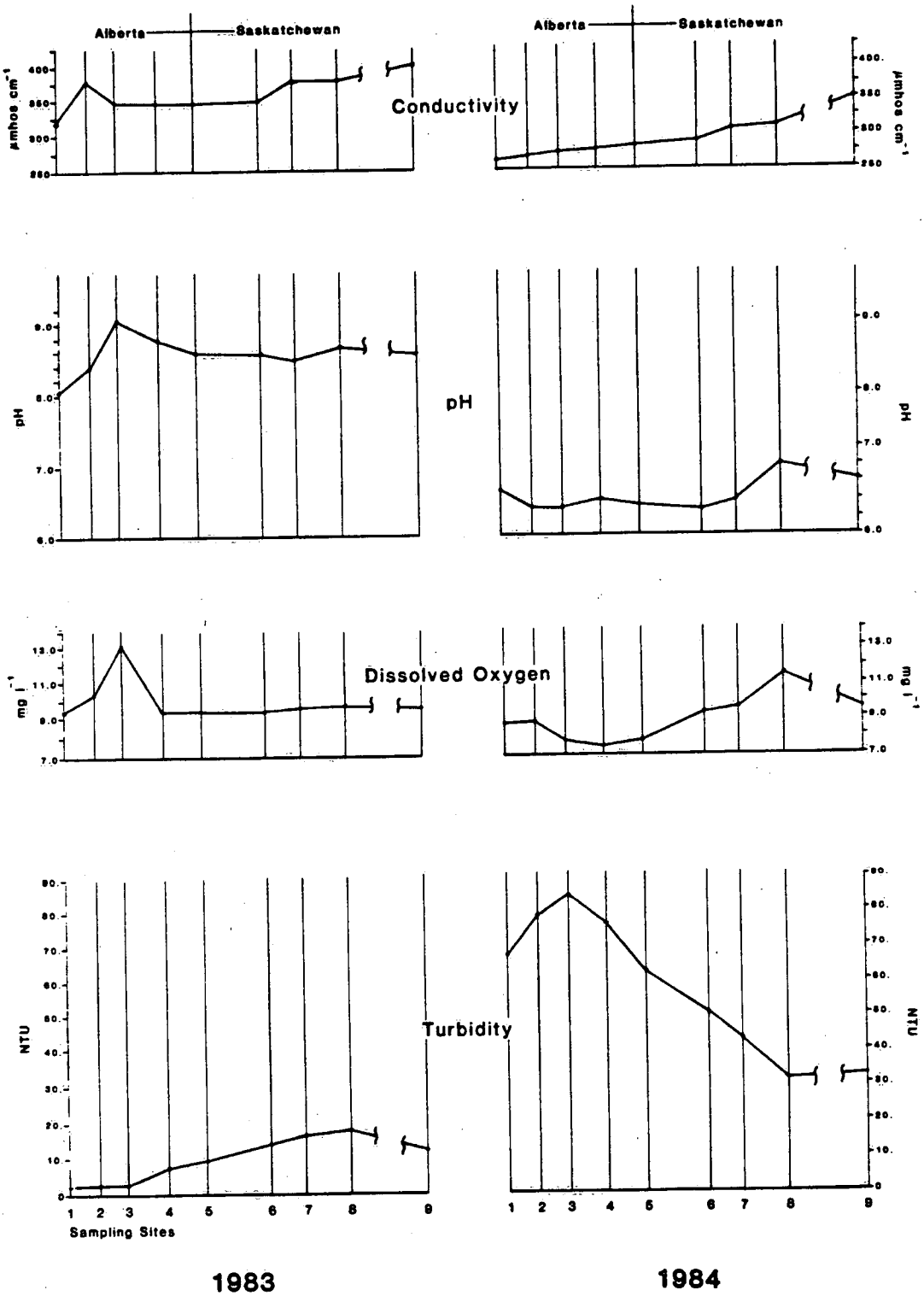

FIGURE 2: Downstream characteristics of water in the North Saskatchewan River in summer of 1983 and spring of 1984.

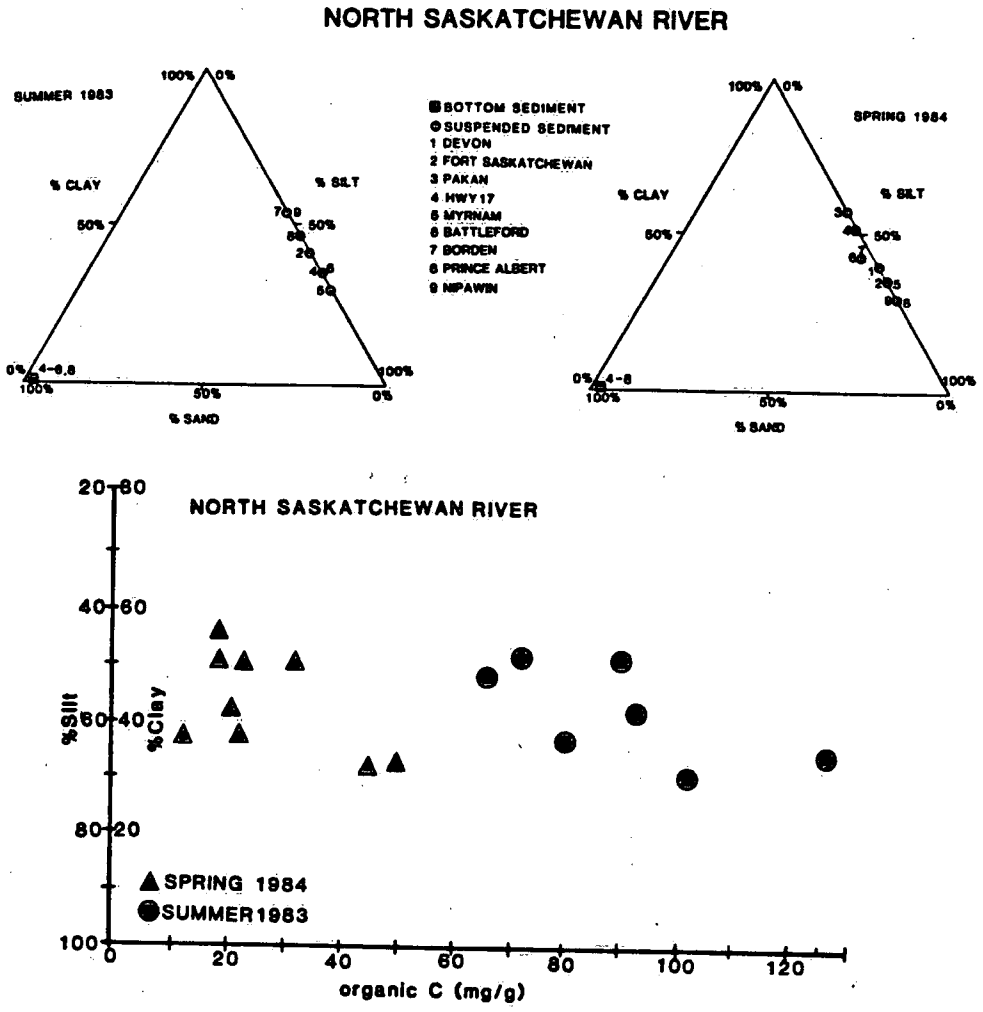

FIGURE 3: Particle size and organic carbon of suspended and bottom sediments for summer, 1983, and spring, 1984, on the North Saskatchewan River.

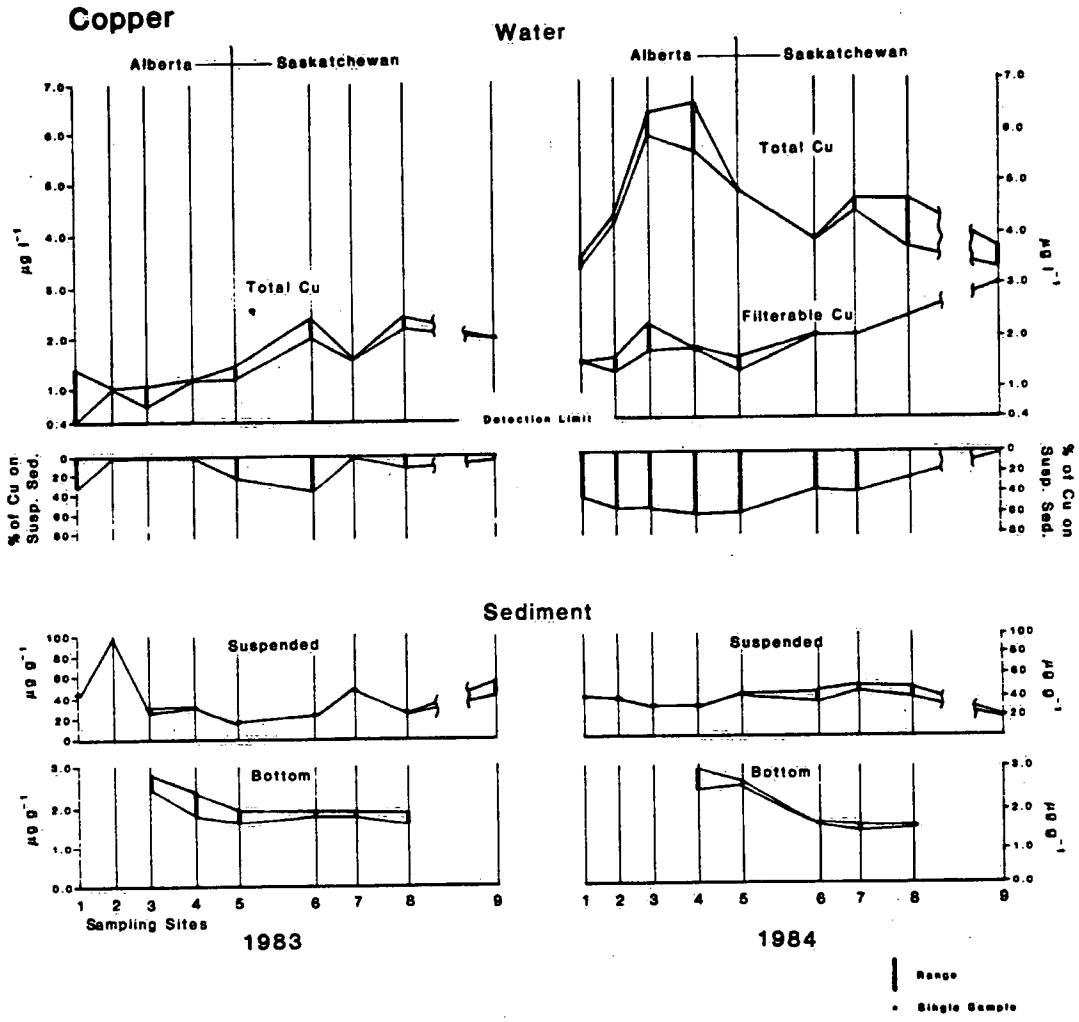

FIGURE 4: Downstream characteristics of mercury and zinc in water and on suspended and bottom sediments in the North Saskatchewan River in summer of 1983 and spring of 1984.

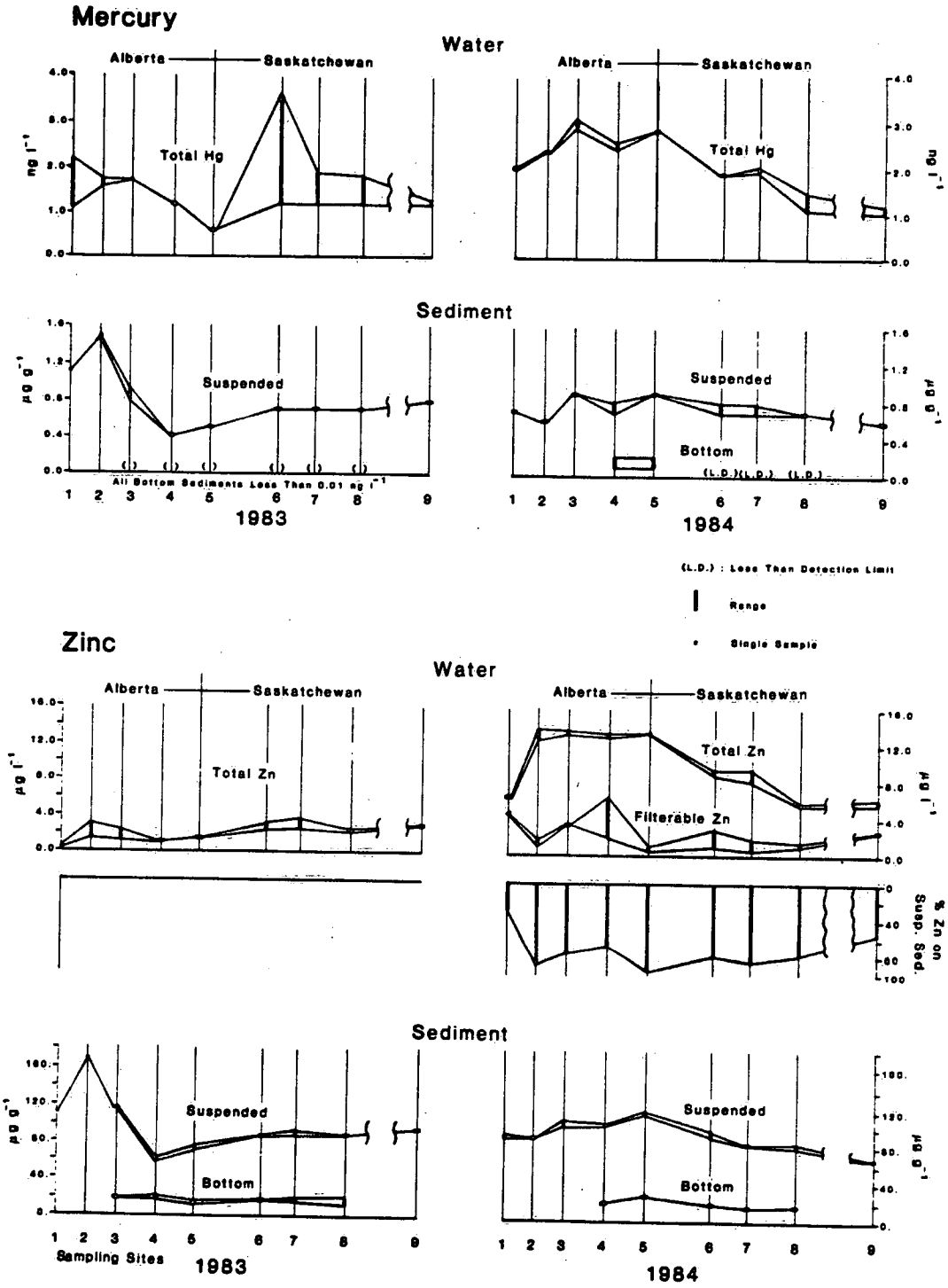

FIGURE 5: Downstream characteristics of mercury and zinc in water and on suspended and bottom sediments in the North Saskatchewan River in summer of 1983 and spring of 1984.

Water and Sediment Chemistry

Levels of Cu, Pb, Zn, Cd, Ni, Cr, Hg, As, Se, Mn, and Fe have been determined for all whole and filtered waters, and for suspended and bottom sediment samples. Results for Cu, and Hg and Zn are presented respectively in Figures 4 and 5.

Whole Water: Summer levels of metals tend to be low, whereas spring high flow (1984) has much higher levels of metals. This reflects the increased amount of suspended sediment in whole water samples taken during spring flow conditions. The sediment concentration - whole water metal chemistry relationship is demonstrated in (1) the parallelism between whole water concentrations in the downstream direction and the trend in turbidity (Figure 2), and the proportion of total metals in water attributed to the particulate phase as shown for Cu and Zn in Figures 4 and 5.

Suspended sediments: Suspended sediments during 1984 high flow are probably unenriched and indicative of background (i.e. eroded soil, bank collapse, etc.). Low-flow concentrations of the three metals on suspended sediments indicate an immediate impact below the city of Edmonton followed by a downward correction over sites 3 - 4, then an upward trend from site 4 (not noticeable for Cu) which parallels the increasing turbidity trend during the summer sampling period. It is not known whether the downward correction below Edmonton represents dilution by sediment addition, as noted by Ongley and Blachford (1984), or net loss of metal from suspended sediments caused by other biogeochemical phenomena. Because the downward correction between sites 2 - 4 during 1983 low flow reaches levels commensurate with spring background, sediment dilution may be effectively reducing the summer metal values on suspended sediment downstream of Edmonton to a concentration commensurate with background. However, increasing summer turbidity as one moves further downstream implies net increase in total transport of metal.

Bottom Sediments: As expected, levels of metals on bottom sediments are much below that found on suspended sediments or, in some situations, below detection levels. Bottom sediments cannot be used to indicate sediment-associated metal trends in the North Saskatchewan River.

Priority Chemicals/Toxicity - 1983

The relative toxicity for each substrate (water, suspended sediment, bottom sediment) can be assessed using the statistical measure of "fitness" for the P.redivivus bioassay. Fitness is a dimensionless statistical value which measures the aggregate effects of lethality, general inhibition, and phenotoxicity, against control samples of the test organism. Fitness varies from zero (very substantial decrease in growth and survival) to 100% (no important response). The fitness statistic is associated with levels of statistical significance; here, significance of effect at the 0.05 level can be achieved for fitness values between zero and 98%. For the purpose of Table 3 we have used "Risk" where

$$\text{Risk} = 100 - \text{fitness}$$

A value of zero implies "no risk", whereas 100 implies "very large risk". Each site has been assigned a risk factor for each of the fractions analyzed. Underlined values are those which are statistically significant at the 0.05 level. An aggregate risk factor for each substrate for each site is obtained by sumation of the individual risk factors. Because the risk factors are dimensionless, relative response between the substrates can be compared. Because the Ames test failed to provide a response to the spiked samples, as noted below, the Ames data have not been factored into risk assessment in Table 3. One or more positive results with the Ames test are indicated by "*" on Table 3. The Ames results were in reasonable agreement with P.redivivus for suspended sediments; agreement was not especially good for water samples.

Priority Chemicals/Toxicity - 1983: Water

A summary of GC/MS analysis for "priority chemicals" obtained for water samples, together with a summary of toxicity data, is shown in Table 4. In NO case was the presence of priority chemicals synonymous with the observed toxicity. Except in one case (Devon) **no priority chemicals were detected** above the GC/MS detection limits reported by US-EPA (Longbottom and Lichtenberg, 1982).

Toxicity was observed for the neutral extracts obtained from Fort Saskatchewan and Highway #17. Toxicity was also observed for the acid extract from Nipawin and Prince Albert by both biological assays. Again, no priority chemicals were detected in these extracts. For many extracts, disparities in response were revealed indicating toxicity to one organism but not the other. Our QC/QA sample demonstrates this point; toxicity was observed with both the neutral and acid extract using P. redivivus but not with the Ames test. Since this water sample was fortified with pesticides at the 5-10 ppb level, one has to question either the use of GC/ECD analysis with ppt detection limits, or question the utility of the Ames test as a screening tool.

Aggregate risk factors (Table 3) for water samples indicate that toxic effect by site is :

Myrn.Br. >Devon >F.Sask. >High.17 >Pakan >Nip. > Pr.A. >Batlfrd. >Bord.Br.

Priority Chemicals/Toxicity - 1983: Suspended Sediment

Findings for suspended sediments are summarized in Table 5. Significant toxicity observed in the acid fraction of seven of the nine sites (Table 3) is confirmed in both biological tests. Priority phenols were found to be present in three out of the seven toxic fractions. Although several priority pollutants were identified in the B/N extracts, no toxicity was observed in both tests, although toxicity was often observed with one organism but not supported by toxicity in the other. This is likely due to sensitivity differences between the test organisms.

Aggregate risk factors for suspended sediment samples indicate that toxic effect by site is :

Bord.Br. =Pr.A. =Nip. >Pakan >Batlfrd. >F.Sask. =Myrn.Br. >Devon >High.17

Note that this ordering is very different than that for water samples.

Priority Chemicals/Toxicity - 1983: Bottom Sediments

Six sites were surveyed for toxic chemicals. Note, however, that the Fort Saskatchewan sample is atypical of bottom sediments at that site. Bottom sediments at Fort Saskatchewan are cobble-sized material. The sample taken was of finer-grained sediment deposited during low water behind a gravel berm.

Although priority chemicals (Table 6) were detected in the B/N extract from several sites, toxicity was not observed for these extracts in both assays. As in previous cases toxicity was observed in one or the other but not in both. Significant response (with 95% probability) occurred in only one fraction at one site (Fraction 4, Fort Saskatchewan).

Aggregate risk factors for bottom sediment samples indicate that toxic effect by site is:

Myrn.Br. > F.Sask. >Pr.A. >Batlfrd. > High.17 = Bord.Br.

Note that bottom sediments cannot be used to predict the chemistry of suspended sediments or of water. This is consistent with the findings for heavy metals here and in other work.

Pesticides

Because of extensive use of agricultural pesticides in western Canada a subset of water samples and suspended sediments were analyzed for pesticides by capillary GC/ECD.

Water: The data are summarized in Table 7. Generally, alpha_BHC and Gamma-BHC were the prediminate pesticides found in the 1-5 $ng.L^{-1}$ (ppt) range. The presence of endrin aldehyde and endosulfate is discounted due to the complex chromatograms. These results will be confirmed by mass spectrometry.

Suspended Sediment: The data are summarized in Table 8. Although the presence of many pesticides is indicated by GC/ECD, this is likely due to interference. Results will be confirmed by mass spectrometry.

TABLE 3: TOXIC RESPONSE : RISK FACTOR FOR 1983 LOW WATER CONDITIONS

	ALBERTA					SASKATCHEWAN			
Site	Devon	F.Sask.	Pakan	Myrn.Br.	High.17	Batlfrd.	Bord.Br.	Pr.Alb.	Nipawin
Fraction									
WATER									
Acid	5*	1*	0	0*	16	0	0*	4	6*
Neutral	25	23	12	35	5*	0	0	0	2
Aggregate Risk	30	24	12	35	21	0	0	4	8
SUSPENDED SEDIMENT									
Acid	7*	35*	14*	13*	16*	41*	100*	100*	100*
B/N 1	0	0	1	0	0	0	0	0	0
2	0	0	2	5	0	0	0	0	0*
3	13	0	12	7	0	0	0	0	0
4	0	0*	16	10	0	0	0	0*	0
Aggregate Risk	20	35	45	35	16	41	100	100	100
BOTTOM SEDIMENT									
Acid		6		0	0	0	0	0	
B/N 1		0*		8	0	0	0	3	
2		1		4	0	2	0	0*	
3		0		0	0	0	0	0	
4		4		0	0	0	0	0	
Aggregate Risk		11		12	0	2	0	3	

Underscore = significant response at 95% probability.
* = response with Ames test.

TABLE 4: SUMMARY OF PRIORITY CHEMICALS AND TOXICITY - WHOLE WATER SAMPLES NORTH SASKATCHEWAN RIVER, 1983

Site	Sample Size	Priority Chemicals	Fraction	Conc. ($ug.L^{-1}$)	Toxicity Ames	Toxicity P.red.*
Devon	16L	nd	acid	nd	Yes	No
		Nitrobenzene	neutral	7.25	No	Yes
Fort Sask.	8L	nd	acid	nd	Yes	No
		nd	neutral	nd	Yes	Yes
Pakan	16L	nd	acid	nd	No	No
		nd	neutral	nd	No	Yes
Myrnam Br.	8L	nd	acid	nd	Yes	No
		nd	neutral	nd	No	Yes
Highway 17	8L	nd	acid	nd	No	Yes
		nd	neutral	nd	Yes	Yes
Battlefords	16L	nd	acid	nd	No	No
		nd	neutral	nd	No	No
Borden Br.	8L	nd	acid	nd	Yes	No
		nd	neutral	nd	No	No
Prince Alb.	16L	nd	acid	nd	Yes	Yes
		nd	neutral	nd	Yes	No
Nipawin	16L	nd	acid	nd	Yes	Yes
		nd	neutral	nd	No	Yes
QA/QC	4L	Yes (Phenols)	acid	6.25 -25.0	No	Yes
		Yes (pesticides)	neutral	2.50 - 9.38	No	Yes

* Significant at the .05 level.

TABLE 5: SUMMARY OF PRIORITY CHEMICALS AND TOXICITY - SUSPENDED SEDIMENTS NORTH SASKATCHEWAN RIVER, 1983

Site	Sample Size (g)	Priority Chemicals	Fraction	Conc. ($ng.g^{-1}$)	Toxicity Ames	Toxicity P.red.*
Devon	21.39	nd	acid	nd	Yes	No
		nd	B/N 1	nd	No	No
		Phenanthrene or Anthrazene	2	70.1	No	No
		nd	3	nd	No	Yes
		nd	4	nd	No	No
Fort Sask.	29.52	4-nitrophenol	acid	610.	Yes	Yes
		pentachlorophenol	acid	237		
		nd	B/N 1	nd	No	No
		Phenanthrene or anthracene	2	84.7	No	No
		Flouranthene	2	67.8		
		nd	3	nd	No	No
		nd	4	nd	Yes	No
Pakan	37.03	nd	acid	nd	Yes	Yes
		nd	B/N 1	nd	No	No
		Pyrene	2	27.0	No	No
		nd	3	nd	No	Yes
		Bis(2-chloroethoxy) methane	4	365.	No	Yes
Myrnam Br.	48.18	nd	acid	nd	Yes	No
		1,4 Dichlorobenzene	B/N 1	72.6	No	No
		nd	2	nd	No	Yes
		nd	3	nd	No	Yes
		nd	4	nd	No	Yes
Highway 17	52.1	nd	acid	nd	Yes	Yes
		nd	B/N 1	nd	No	No
		nd	2	nd	No	No
		nd	3	nd	No	No
		nd	4	nd	No	No
Battlefords	50.86	2,4-Dimethylphenol	acid	393.	Yes	Yes
		nd	B/N 1	nd	No	No
		Fluoranthene	2	9.83	No	No
		nd	3	nd	No	No
		nd	4	nd	No	No

TABLE 5 continued.

Borden Br. 50.71	nd	acid	nd	Yes	Yes
	Phenanthrene or Anthracene	B/N 1	19.7	No	No
	1,4-Dichlorobenzene	1	118.		
	Lab accident	2	--	--	--
	nd	3	nd	No	No
	Isophorone	4	355.	No	No
Prince Albert	Pentachlorophenol	acid	576.	Yes	Yes
46.03	nd	B/N 1	nd	No	No
	Phenanthrene or Anthracene	2	21.7	No	No
	nd	3	nd	No	No
	nd	4	nd	Yes	No
Nipawin 25.85	nd	acid	nd	Yes	Yes
	nd	B/N 1	nd	No	No
	nd	2	nd	Yes	No
	nd	3	nd	No	No
	nd	4	nd	No	No

* Significant at the .05 level.

TABLE 6: SUMMARY OF PRIORITY CHEMICALS AND TOXICITY - BOTTOM SEDIMENTS
NORTH SASKATCHEWAN RIVER, 1983

Site	Sample Size (g)	Priority Chemicals	Fraction	Conc. ($ng.g^{-1}$)	Toxicity Ames	P.red.*
Fort Sask.	61.72	nd	acid	nd	No	No
		1,4-Dichlorobenzene	B/N 1	89.1	Yes	No
		Phenanthrene or Anthracene	1	64.8		
		Naphthalene	1	267.		
		nd	2	nd	No	No
		nd	3	nd	No	No
		N-nitroso-di-n-propylamine	4	219	No	Yes
Myrnam Br.	53.67	nd	acid	nd	No	No
		nd	B/N 1	nd	No	No
		nd	2	nd	No	No
		nd	3	nd	No	No
		nd	4	nd	No	No
Highway 17	57.67	nd	acid	nd	No	No
		Pyrene	B/N 1	8.67	No	No
		1,4-dichlorobenzene	1	86.7		
		nd	2	nd	No	No
		nd	3	nd	No	No
		nd	4	nd	No	No
Battlefords	57.43	nd	acid	nd	No	No
		nd	B/N 1	nd	No	No
		nd	2	nd	No	No
		nd	3	nd	No	No
		Isophorone	4	2995.	No	No
Borden Br.	56.05	nd	acid	nd	No	No
		1,4-Dichlorobenzene	B/N 1	107.	No	No
		nd	2	nd	No	No
		nd	3	nd	No	No
		nd	4	nd	No	No
Prince Albert	51.68	nd	acid	nd	No	No
		nd	B/N 1	nd	No	No
		nd	2	nd	Yes	No
		nd	3	nd	No	No
		nd	4	nd	No	No
Method Blank		nd	acid	nd	control	
		nd	B/N 1	nd	control	
		nd	2	nd	control	
		nd	3	nd	control	
		nd	4	nd	control	

* Significant at the .05 level.

TABLE 7: PESTICIDES IN WATER -- NORTH SASKATCHEWAN RIVER, 1983 ($ng.L^{-1}$ = ppt)

PESTICIDE	SAMPLE SIZE(L)	ALPHA BHC	GAMMA BHC	DELTA BHC	BETA BHC	ENDOSULF I	4,4'DDE	ENDOSULF II	ENDRIN ALD.	ENDOSULF SULFATE
Devon	16	5.1								
Fort Sask.	8	3.4								
Pakan	16	2.4								
Myrnam Br.	8	5.2	1.1							
Highw. 17	8	3.9	1.2							
Battlefords	16	4.4	0.85						13.7	3.59

TABLE 8: PESTICIDES ON SUSPENDED SEDIMENT -- NORTH SASKATCHEWAN RIVER, 1983 ($ng.g^{-1}$ - ppb)

PESTICIDE	ALPHA BHC	GAMMA BHC	BETA BHC	DELTA BHC	ALDRIN	ENDS I	HEPT EPOX	DDE	DDD	DIELDRIN	ENDRIN	HEPTA CHLOR
Devon					1.2	0.24	0.28	8.7	0.61	0.23	1.9	
Fort Sask.	0.92	1.7	1.8	1.42	16.3	5.0	2.1	3.6	3.4	4.75	7.4	2.9
Pakan	0.89			0.26			0.68	2.5	1.22	0.49		
Myrnam Br.	0.44				0.05							
Highway 17	0.75					0.81						
Battlefords	0.67	0.33			0.09	0.96	0.26	1.6	0.85			

CONCUSIONS

The chemistry of metals is typical of prairie rivers with high concentrations in whole water samples during high water and low concentrations at reduced level of flow. Suspended sediment is enriched immediately below the metropolitan area of Edmonton, however concentrations decline downstream to levels commensurate with background, possibly due to dilution by unenriched sediment inputs into the system. Bottom sediments are impoverished in metals relative to suspended sediments and cannot be used to indicate downstream trends either for water or suspended sediment metal chemistry.

Organic chemistry, combined with two independent bioassays - the P.redivivus and Ames tests, lead to critical questioning of the value of current priority chemical screening practices for environmental sensing purposes. Our observations are especially germane as the data should reflect the influences of point sources.

Although pesticides were detected in all water samples at trace levels (1-5 ppt), priority chemicals were detected at only one site; this extract was not toxic. Toxicity was observed for only one water sample but no priority chemicals were detected.

The most toxic responses were observed for the acid fractions in the suspended sediments. Seven of the nine sites sampled showed toxicity in both bioassays. Priority phenols were found in three of the seven toxic fractions; no priority chemicals were detected in the remaining four toxic fractions.

There is little comparability between toxic response of whole water samples and suspended sediments contained in the whole water samples.

No conclusive toxicity was observed in the bottom sediment samples.

The following questions arise, therefore:

1. Toxic response in the absence of Priority Chemicals implies that there may be profound deficiencies with the menu-driven approach to environmental sensing of toxic chemicals.

2. The much greater toxic response and larger number of priority chemicals associated with suspended sediments implies that water may be an inadequate medium upon which to base toxic chemical criteria for toxic chemical sensing purposes in aquatic systems.

3. Our data suggest that certain priority chemicals which exist in water or on sediments, produce no toxic response in one or both of the bioassay procedures.

4. Routine chemical screening of water samples for organic compounds is expensive, generally produces ND values, misses not only many toxic compounds which are associated with suspended sediment but also those compounds which are not part of the screening menu, and can provide no ecologically relevant information for those compounds.

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ADDENDUM

The following comments were received from Dr. P.J. Blackall, Director of Scientific Programs Branch of the Environmental Protection Service (Western and Northern Region). Dr. Blackall is responsible for the laboratory which produced the Ames data for this study. Dr. M. Samoiloff, an aquatic toxicologist at the University of Manitoba and a participant in this study, has provided a response which is reproduced in the following pages. The report has not been changed as I believe Dr. Samoiloff answers the comments raised by Dr. Blackall. Nevertheless, this dialogue raises several interesting points concerning the use of the Ames test for ambient water quality purposes. Any additional dialogue on the subject will be reflected in the final report on this study.

E.D. Ongley (for authors)

COMMENTS BY DR. P.J. BLACKALL

1. "The reference for the Ames test is not correct and should be as per memo of June 26, 1984 (Elliot to Birkholz)."

2. "The Ames test assess (sic) mutagenisity and is not designed to quantify toxicity. Therefore, any such reference is incorrect. Knowledgeable readers would realize the inappropriate use of the Ames testing protocol."

3. "The report does not present the mutagenisity data as developed by G. Elliot."

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COMMENTS ON BIOASSAYS USED IN THE

NORTH SASKATCHEWAN RIVER STUDY - 1984

by

Martin R. Samoiloff

30 July, 1985

INTRODUCTION

The only method for determining the actual toxic potential of a complex environmental sample is by the use of a bioassay to establish the actual toxic effects of the complex mixture. An appropriate indicator organism is exposed to a standard dilution of the tested fluid, and the effects on some specific biological endpoint are evaluated. In this fashion, the question of whether or not the sample fluid is toxic can be resolved.

There are three broad types of toxic effects; lethality, inhibition of one or more physiological processes, and long-term, low-frequency effects, such as mutagenesis, carcinogenesis, or the generation of developmental abnormalities. Generally, it is considered that materials with lethal effects at environmental levels are much more serious than those with inhibitory effects, especially if the specific effects are relieved as exposure is reduced. Long term effects, for the most part, have very little impact on natural biological populations, but are of serious concern to human health.

It is possible, therefore, to establish a ranking of toxic effects. Such a ranking, in order of decreasing toxic importance to natuaral populations, would be:

1. Death of all or a significant proportion of a test population.

2. Irreversible damage to one or more physiological processes required for normal functions.

3. Reversible damage to one or more physiological processes required for normal functions.

4. Long term effects with little overall impact on the population as a whole.

Most bioassays have been developed to detect only one of these types of effect as their biological endpoint. For example, there are numerous tests using various organisms (Daphnia, fish, algae) to detect lethality. The short-term effects of lethality or inhibition are relatively easily measured. Lethality is normally measured by determining the number of test animals that die within a specific test period, or by the exposure time that is required to kill a specific proportion of the population of tested organisms.

Inhibition is normally measured by determining the reduction of some activity of the test organism following a fixed period of exposure to the tested material, or by detecting the exposure time required to produce a specific reduction in some specific endpoint activity. Generally, tests for inhibition also detect lethality, while tests for lethality usually ignore "sick" individuals in the test population.

Tests for mutagenesis and carcinogenesis must be more complex than bioassays for the short-term effects. Mutagenesis is a rare event; the best estimate of the spontaneous mutation rate in man and most animals is 2 to 3 mutations per million gene loci ("genes") per generation. The most powerful know mutagens will induce an average of approximately 2 mutations per 10,000 gene loci per generation, a hundred-fold increase over the spontaneous frequency, but overall, only a .02% frequency. Tests for mutagenesis must, therefore, examine a large number of target gene loci.

Because mutation is a rare event, and because there is no evidence that mutagenesis poses any risks to natural populations, test for mutagens have been developed primarily to evaluate many consumer products and workplace and environmental contaminants in a human public health context, rather than for the assessment of potential environmental damage.

One widely used test for mutagenesis was developed by Bruce Ames and co-workers in the mid-1970s. This "Ames test" exploits strains of the bacterium Salmonella typhimurium containing known mutations that block the synthesis of the amino acid histidine, which is required for growth of this bacteria. Large populations of the bacteria, containing up to a billion cells, are exposed to the tested material, and then placed on a plate that effectively lacks histidine. Since the cells carry a mutation blocking the synthesis of histidine, most cells will not grow. However, if a mutation occurred that "corrects" the original mutation, cells carrying the reversion will divide and produce colonies of cells. A count of the number of such revertant colonies will, therefore, reflect the number of mutations that corrected the mutation at the histidine gene locus, and indicate the mutagenicity of the tested material.

The Ames test has been primarily used for the evaluation of products and the determination of the mutagenic properties of individual chemicals. The Ames test is usually used in a semi-quantitative fashion, catagorizing materials as either slightly, moderately, or highly mutagenic within a specific concentration range.

At present, the evidence indicates that agents that cause mutation also increase the risk of cancer. Therefore, tests for mutagenesis are also considered tests for carcinogenesis. Very often, materials that are not themselves mutagens are chemically modified by some biochemical process, undergoing conversion to a

mutagenic substance. The Ames test detects the conversion of these "promutagens" to mutagens by treating the tested material with an extract of oxidizing enzymes obtained from rats. This extract, termed S9, is required since the bacteria cannot carry out these conversions.

There are several advantages of the Ames test. The test is rapid, providing indications of mutagenic action within a 3 day test period. Because the Ames test examines a large number of cells, it is highly sensitive to mutagens. The test is relatively inexpensive, being much less costly than similar tests using whole laboratory animals such as the rat. Typically, in Canada, a complete Ames test using four different tester strains, with and without S9, costs $1000, while tests with rats would cost approximately 10 times this amount. In the licensing of many products, required by law prior to marketing, a series of health and safety criteria must be met. Often the Ames test is used as the preliminary toxicity test, as a cost-effective first-step, prior to subsequent, and far more expensive testing.

The Ames test was established as a rapid , relatively cost-effective test for mutagens, it is not normally used to detect the more immediate toxic effects such as lethality or inhibition. For examining the overall toxicity of a material for licensing or regulatory purposes, it is one of a suite of tests, each focusing on a specific toxic endpoint, that collectively are used to establish the toxicity of a tested material.

There is an important distinction that must be made between toxicity tests used in the licensing and regulatory processes, directed towards finding the toxicity of specific compounds or products, and the toxicity tests used for the ranking of the relative toxic impact of a series of complex samples. In tests for licensing purposes, the basic question that is asked is:

> "At what concentration does the tested material produce a detectable toxic effect?"

This information is used to determine if the product can be marketed, and is often used to establish the "safe-levels" of the material, by the application of some correction factor to the minimum concentration with detectable effect.

On the other hand biological tests for evaluating the toxic potential of a series of complex environmental samples focus on a totally different question:

> "Which of this series of samples poses the greatest, immediate risk to exposed populations?"

The thrust of this approach, termed a "yardstick" approach by Samoiloff and Wells (1984), is to use one or more biological tests in the laboratory as a method for the evaluation and the

ranking of a series of highly complex contaminated environmental samples.

The "yardstick" approach requires the use of simple cost-effective biological indicator systems that are can detect one or more biological endpoints of ecological importance. This type of approach is not directed towards the registration of a consumer product, or the establishment of "safe-levels" of a single type of environmental contaminant but, is directed towards the ranking of the toxic potential of complex environmental samples.

One test, developed for use in this "yardstick" approach, uses the free-living nematode Panagrellus redivivus. This simple multicellular animal has a five day generation time, and genetic stocks have been developed with increased responses to toxic materials. The life-cycle of this animal is quite simple. After embryonic development, the animal grows through three juvenile stages to an adult stage. Each stage occurs within a specific size range, so that each stage can be readily recognized.

For the biological assay, a known number (usually 100) of animals at the earliest stage are placed in the test material, and the growth and survival of this test population determined after a 96 hour growth period. Net lethality is determined by counting survivors at the end of the test period, and comparing this with the number of survivors in an unexposed population. Growth is determined by counting the number of animals that have completed one, two or all three larval stages, compared to growth in the unexposed control population. Inhibition of early growth is an indicator of inhibition of physiological processes. The final molt requires extensive gene activity, and inhibition of this molt indicates damage to the genetic system, which might be due to mutagenesis, but can also be due to other effects at the gene level. Known mutagens selectively inhibit this final stage of growth.

The Panagrellus bioassay, therefore, provides quantitative information on three different biological endpoints (survival, growth and maturation), making it a good test for "yardstick" use.

The Ames test can also be used as in "yardstick" analyses. If the tester stocks are exposed to standard dilutions of tested material, one of three results can be observed:

1. The cells will grow until residual histidine is all utilized. This case indicates no biological effect.

2. The cells will be unable to grow although there is some residual histidine present. This case represents "cell-death" or cytotoxicity.

3. Some cells will undergo reversion, and divide to form colonies. This case represents mutagenesis.

BIOASSAYS IN THE NORTH SASKATCHEWAN RIVER PROJECT

The justification for performing the bioassays used in the North Saskatchewan River Project was to use the results of the bioassays to evaluate the toxic potential present in various components of the water column, suspended sediments, and bottom sediments. The procedure was to send blind-labeled extracts to two different labs for evaluation. One lab performed the Ames test, while the other performed the Panagrellus bioassays. Since the objective of the study was to compare the risk potential of the submitted samples, an important component of the bioassay protocol is to ensure that the bioassays are performed with standard concentrations of tested extract. All nematode tests were performed at one standard dilution, ensuring that there was comparability between the extracts.

In testing consumer products or contaminant that could pose a potential human health risk, the Ames test is performed in a very standardized fashion. Several criteria must be met in such Ames tests:

1. A series of concentrations of the tested material are evaluated, and tests are primarily performed at that concentration range in which the bacteria reproduce. Obviously, a test evaluating a material only for mutagenesis requires that the bacteria be capable of reproducing. Often high concentrations of a tested material kill the cells, so lower concentrations are required to meet this criterion.

2. In order to be considered as a mutagen, the tested material must show a dose-response pattern of mutagenesis. The tested material should show greater frequencies of mutagenesis at higher concentrations of tested material in the concentration range select under the criteria established above.

The above two criteria are required for tests of products and materials in order to establish safe levels of the materials. The results of such tests provide information that the tested material is mutagenic in a specific concentration range. This is not the information required in the North Saskatchewan River project.

In the present study, the Ames test is used as a one of two methods used to evaluate the relative toxic potential of a series of samples, not to establish safe levels. It is most important that this objective be borne in mind. In the context of this

project, the Ames test is used as a ranking tool, not as a means for the evaluation of safe levels. Therefore, since comparisons between samples require that standardized concentrations be used, criterion 1 above, used for the determination of the mutagenicity of a product or for establishing safe levels of that material, are not applicable.

When performed at standard concentrations, a particular Ames test can give one of three results; the sample may be non-toxic, cytotoxic , or mutagenic. Obviously, cytotoxicity (cell death or an inability to divide) is a much more ecologically significant toxic effect than mutagenesis, in which the cells can divide, but a small portion of the population undergos mutation. The Ames test, used in this fashion, provides an indication of the toxic potential of the tested sample, using two biological endpoints (cytotoxicity and mutagenesis).

The method of Ames testing used in this study is, without question, scientifically valid, and best meets the objectives of the study.

COMMENTS ON THE DRAFT FINAL REPORT

The question has been raised that there could be serious misinterpretation of the Ames test data presented in this report. I consider this unlikely for the following reasons:

1. References to the Ames test (page 12) refer to the methods used as those presented by Samoiloff et al, 1983. In this refereed paper (Environmental Science and Technology 17:329-334), the exact same methods are reported as were used in the present study. This approach, therefore, has already been accepted by the peer-review process.

2. The publication cited above explicitly states that the Ames tests were run at standard dilutions of extract and that cell lethality and mutagenesis were used as biological endpoints. There can be no grounds for misinterpretation.

3. The summary data presented in Table 3 presents numerical data obtained from the Panagrellus bioassay, and an "*" indicating the detection of toxicity by the Ames test. The nematode bioassay is quantitative, the Ames test is not. The summary data in Tables 4-6 present the bioassay data integrated with the search for toxic chemicals. This is the actual data, leading directly to the conclusions as presented.

The only possible misinterpretation could arise if a reader of this data, unaware of the earlier report by Samoiloff et al concluded that te Ames test data presented in Tables 3-6 was strictly mutagenesis. For this eventuality, a brief comment on each Table, or as a brief addendum, could be included stating:

> "In most cases, toxicity detected by the Ames test consisted of an inability of one or more testor strains to undergo normal divisions at low histidine concentrations in one or more standard dilutions of extract, and should be interpreted as cytotoxic rather than mutagenic effects."

4. The design of the project was such that the bioassays were performed on blind-labeled samples, with bioassays performed in a routine fashion. There is neither a need nor any justification for any significant input by those performing the bioassays on the other aspects of the design or interpretation of the results. The data input from the individuals carrying out the bioassays is most straightforward, "sample number X produced effect Y".

5. The statement is made on pages 18 and 20 that the Ames test failed to detect spiked samples. If this is the case, it must be reported, and cannot be altered after the fact. The use of spiked samples is important for quality control, and is indicative of good research. Failure to detect the spiked samples suggests a low sensitivity of the Ames test, as applied in this study and must be considered. This result suggests that the nematode bioassay is more applicable to the type of problem addressed by the present study.

6. The results suggest that the Ames test is not a good method for evaluating the overall risk potential of complex environmental samples. This is probably a correct conclusion for several reasons:

   a. the Ames test is expensive for screening large numbers of samples.

   b. the Ames test, even as modified for these studies, provides only limited toxic endpoints, focusing on the least ecologically significant effect (mutagenesis).

c. the Ames test, while superb for examining products and public health risks, is not overly applicable for ranking toxic effects of a series of environmental samples, as it is only semi-quantitative and is not highly sensitive for either lethal or inhibitory effects.

Neither the scientific validity of the results presented nor the correctness of the conclusions drawn from the data can be called into question the grounds of the bioassay data.

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