Concepts for developing Canadian water quality guidelines for effluents from bleached pulp mills

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Summary

The objectives of this report are to review information that is relevant to deriving water quality guidelines for bleached pulp mill effluent (BPME), to identify chemicals of particular concern, and evaluate methods for developing guidelines and other tools for interpreting the potential environmental effects of mixtures.

(a) ***Existing Canadian water quality guidelines*** *for single chemicals are relevant for waters affected by BPME and should be used. Guidelines for dissolved oxygen, pH, and phenols should be revised.*

(b) ***Toxicity data for chemicals in BPME*** *have been surveyed by Environment Canada and other groups. Environment Canada is currently summarizing existing data, to support the development of water quality guidelines and identify areas of deficiencies in information.*

(c) ***A guideline based on body burden of each persistent bioaccumulative toxicant*** *is a desirable single-chemical approach for those lipophilic toxicants which tend to partition into ecosystem compartments other than water. Environment Canada is currently developing, for the Canadian Council of Ministers of the Environment (CCME), a method for deriving tissue residue guidelines for protecting wildlife which consume aquatic organisms. Dangerous bioaccumulative and persistent toxicants are not evident in BPME, however, except for chlorinated dioxins and furans.*

(d) ***"Toxicity Equivalents"*** *(TEQs) are widely accepted for expressing mixtures of chlorinated dioxins and furans as a single number, in terms of equivalent amounts of the most toxic isomer. This system is available for chlorinated phenolics in BPME, appears to be successful, and could be set up for resin and fatty acids, the other main toxicants. This method of expressing mixture toxicity is essentially the same as item (e).*

(e) ***The "toxic units model" for mixtures*** *assumes simple addition of toxic effects of chemicals in a mixture, within categories of similar chemicals and even between categories. There is appreciable evidence that this is approximately true at the sublethal level, although less-than-additive effects are common, i.e. the toxic units approach is somewhat over-protective. Some such system should be developed to work with the mixtures in effluents by starting from chemical measurements, because single-chemical guidelines cannot be relied upon to protect aquatic organisms when a mixture of substances is present.*

(f) ***A water quality guideline in terms of toxicity*** *could be defined for BPME, by specifying a sublethal toxicity test (or tests) and a safety factor. Specifying the procedure would provide the uniform national guideline for surface waters. To use that guideline as a local water quality objective, the sublethal test(s) would be done on the effluent, and the objective would be stated as a percent concentration of BPME in the receiving water, thought to be without deleterious effects. The numerical value of the objective would vary with the particular mill and the particular time. I recommend setting up a water quality guideline for effluents in surface water, based on direct toxicity testing. Such direct use of toxicity should be accurate, effective, and economical, as an alternative or additional approach to predicting mixture toxicity from a chemical basis (items d and e).*

(g) **Biological techniques** are moving ahead strongly in other places, and Canada no longer appears as a leader in direct use of biological tests. The U.S.A. and Australia have now essentially defined sublethal toxicity testing as a water quality guideline as recommended in item (f). Britain plans to have an environmental quality guideline defined in terms of a biotic index for invertebrate fauna. A site-specific quality objective would be derived by specifying an index for quality of biotic community, which would thus function in a planning mode in similar fashion to a chemical water quality objective.

(h) **Quantitative structure-activity relationships** (QSARs) can greatly assist in predicting relative toxicity of substances, their mode of action and bioaccumulative potential. QSAR should be used for predicting which substances in BPME are most worthy of attention as potential toxicants.

(i) **Modelling** can bridge parts of the process of guideline development, particularly body burdens and environmental fate. Modelling including QSAR can be used in a variety of roles supporting development of guidelines, including those for BPME in water. Use of modelling in a primary role to select guidelines would seem premature. However, British Columbia is using modelling in an attempt to develop site-specific controls for BPME components in food organisms, from the point of view of protecting human health.

(j) The following items are accordingly suggested for serious consideration and possible use in developing guidelines for BPME: classical single-chemical guidelines, both existing and future; tissue residues of single persistent bioaccumulative substances; residues of relatively non-persistent narcotic-type chemicals, probably considering total residue (mixtures of chemicals); mixture toxicity following the toxic units model; direct sublethal testing of effluent with a safety factor; and modelling/QSAR in a supporting role for all activities. These are not mutually exclusive topics.

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1 Introduction

Under the Canadian Environmental Protection Act (CEPA), the Minister of Environment is required to develop environmental quality guidelines for substances such as those on the Priority Substances List. The Minister recently declared effluents from pulp and paper mills using bleaching to be "toxic" according to CEPA Part I, Section II. Subsequently, new Canadian Pulp and Paper Effluent Regulations have been implemented. It is the responsibility of Environment Canada, in conjunction with the Canadian Council of Ministers of the Environment (CCME), to develop environmental quality guidelines for use in environmental programs.

Standard and successful methods are available for developing water quality guidelines for individual chemicals (CCME 1991). The guidelines are interpretive tools for designating goals and evaluating risks of elevated levels of specified contaminants in surface waters. They are intended for use along with other tools such as (a) toxicity testing which is another predictive technique as well as a monitoring tool, (b) sediment quality guidelines and tissue residue guidelines, and (c) follow-up audits of the well-being of ecosystems as in Environmental Effects Monitoring under the Pulp and Paper Effluent Regulations (Canada Gazette). These tools have roles that differ appreciably from each other at the conceptual and practical levels, in any broad program of controlling water pollution (Appendix B).

Many Canadian surface waters receive complex effluents such as those from pulp mills. It is a considerable technical challenge to develop guidelines for concentrations of effluent in receiving water, or for mixtures of substances with differing degrees of toxicity. Effluents would create such mixtures in surface waters, but components would vary from mill to mill and time to time. Single-chemical objectives might be met, without necessarily being protective against mixtures of substances in an effluent. That is why potential approaches for predicting effects of chemical mixtures in surface waters are being assessed by Environment Canada.

1.1 Definitions

Certain terms are used in this report in the way that they are defined in the book of Canadian water quality guidelines (CCREM 1987).

Criteria (singular, criterion): scientific data evaluated to derive the recommended limits for water uses. [Certain direct quotations in the present report show the word *criterion* being used with a meaning similar to that defined below for *guideline*. Since that usage may prevail in other organizations or other jurisdictions, it sometimes appears in the present report when words are directly cited from those organizations.]

Water quality guideline: a numerical concentration or a narrative statement recommended to support and maintain a designated water use.

Water quality objective: as guideline, but established to support and protect the designated uses of water at a specified site.

Water quality standard: an objective that is recognized in enforceable environmental control laws or regulations of a level of government.

CCREM (1987) and other agencies list various water uses such as drinking water supply, or recreation, but the present report is concerned with aquatic organisms, i.e. conditions relevant for maintenance, protection and enhancement of aquatic communities and ecosystems.

1.2 Objectives and scope of this report

This report is concerned with effluents from pulp mills with bleaching (Bleached Pulp Mill Effluent or BPME). The general purposes are to: (1) identify in BPME, the chemicals that are suitable for development of single-chemical guidelines; and (2) evaluate approaches for developing water quality guidelines for BPME, recommending useful and scientifically valid ones. From this assessment, it is anticipated that tools will be developed for assessing effects of mixtures, to be used in addition to single-chemical guidelines, to better evaluate risks of contaminants to aquatic ecosystems.

1.2.1 Coverage and format

This report is intended to be at a conceptual level. Much of it concerns the second objective listed above, so it required identification and evaluation of approaches for developing effluent guidelines. Further detailed work would be required to actually develop guidelines.

The report attempts to convey the main points early, then support them with further detail in the following text. A series of **Summaries** is placed in the sections and sub-sections, to give the main findings, in hope of conveying an overall view of the findings, conclusions and recommendations. Sections 1 to 5 cover all the topics and give some explanation of the information that led to the conclusions and recommendations. **A series of appendices** provides reviews, rationales, and further details of the topics. Most of the appendices have titles similar to sections in the main body of the report, for easy identification, and they sometimes repeat information (in more detail) from the main body of the report since they are intended to be complete in themselves. References to **sections, figures, or tables in the appendices** are given in the main body of the report, and will be evident from the letters A to K used to name the appendices. **Literature references** may be omitted in the front part, but are in the appendices.

1.3 Interviews with experts

Twenty experts were interviewed in person or by telephone, specifically for this project (Appendix A, Sprague 1992). These people had special knowledge of BPME, or of recent advances in water pollution control.

The interviews were intended to provide opinions and ideas on approaches that might be adopted, for developing water quality guidelines for BPME. European contacts were made, to take advantage of any innovative approaches from overseas. The chief impression was of much stronger use of biological guidelines in Europe, compared to Canada.

2 Single-chemical guidelines

2.1 Existing water quality guidelines, relevant to BPME

Summary. The existing Canadian water quality guidelines should continue to be used to develop objectives for waters affected by pulp mill discharges. There are a dozen guidelines for general conditions and specific toxic substances that are very relevant to pulp mill effluents. The existing guidelines for dissolved oxygen, pH, and total phenols should be revised to reflect current information.

Existing Canadian water quality guidelines (CCREM 1987) should continue to be used for bleached pulp mill effluent (BPME). Where the guidelines have been used to develop objectives for bodies of water, the **individual objectives should be met** in the waters receiving BPME.

Water quality objectives, derived from guidelines and stated as chemical concentrations in the receiving water, represent a strong tool for water quality management. Their powerful and unique role is in a predictive mode to set limits in receiving water. Satisfactory discharge levels may be planned and waste treatment facilities designed accordingly. This "instream water quality" approach is the second of three tactics that constitute an overall strategy for water pollution control (Appendix B). Chemical monitoring can be used as part of the same tactic, to see if concentrations in the receiving water are within the objectives. Such chemical surveys are often advantageous and convenient, although they do not represent definitive monitoring of whether effects have occurred, since water quality limits only predict effects. Therefore, environmental effects monitoring by some form of biological survey is required as a specific tactic within an overall management strategy.

BPME contains substances for which no guidelines exist and the present bank of toxicity data may not be sufficient to develop defensible new Canadian water quality guidelines for most of the substances in BPME, according to a study by Leach (1991, see also section D.2). Relevant guidelines that already exist include **total suspended solids, chlorine**, certain **metals**, and **ammonia**. The guidelines for categories of **chlorinated phenols** are of particular relevance to BPME.

The guidelines for **dissolved oxygen** and **pH** should be re-evaluated, and they provide particularly difficult technical challenges. Both of these variables are integral qualities of surface water which vary naturally, and not all natural levels are optimal for all organisms. That creates a difficulty in meeting the mandate of the Canadian Council of Ministers of the Environment (CCME) to develop guideline values that are protective of all species. For dissolved oxygen, the recommendations of Davis (1975) should be considered. Davis resolved a major difficulty by providing sets of oxygen levels for different degrees of protection, and his maximal degree might meet the needs of a guideline. Similarly, pH guidelines should be considered in light of systems designed elsewhere, which incorporate degree of change from natural levels.

For **phosphorus** and other nutrients, guidelines currently under development by Environment Canada will be useful and relevant to some situations of pulp mill discharges. The existing guideline for **total phenols** is for relevant substances but is based on inappropriate assumptions and should be raised.

2.2 Toxicity of BPME and its major chemical components

The overall toxicity varies considerably for whole effluent from pulp mills that bleach, as might be expected. Although there are hundreds of substances in BPME, the appreciable sources of toxicity are probably limited to 30 to 50 chemicals, with most of the toxicity accounted for by a dozen substances (supporting information for this section is given in Appendix D).

2.2.1 Toxicity of whole effluent

Summary. Untreated kraft mill effluent may be non-lethal in acute tests, or it may be quite toxic with LC50s as low as 3%, but an historical average LC50 would be in the vicinity of 16%. Sublethal effects of untreated effluents would be expected only at concentrations greater than about 1/20 of the LC50, which would often be about 1% or 2% effluent. Well-treated effluent should cause sublethal effects only at concentrations above 1%, often much higher such as ≥10% effluent.

The lethal and sublethal toxicity of BPME have recently been reviewed by Environment Canada (Jones et al. 1991) and in the background report produced for that Environment Canada document (Sprague and Colodey 1989), so there is no need for an extensive review here. The toxicity of BPME can be expected to change in the early and mid-1990s because of modified processes in the mills and secondary treatment of effluent, changes brought about by a combination of market forces, pressures of government regulations and environmental groups.

The acute lethal action of BPME was extremely variable from mill to mill, up to the late 1980s. About one quarter of Canadian bleached pulp mills had non-lethal effluents (Jones et. al. 1991). Median lethal concentrations have ranged, however, from levels of >100% down to 3%, and the geometric mean LC50 has been estimated as about 16% effluent (Sprague and Colodey 1991). Secondary treatment can remove acute lethality, but if the facilities are not properly operated, the effluent can be quite toxic. Bonsor et al. (1988) provide an Ontario example in which two kraft mills with secondary treatment discharged effluents that were more toxic than the mean toxic concentration of six kraft mills that did not have secondary treatment.

At the sublethal level, bleached pulp mills showed major differences between untreated and treated effluents, up to the late 1980s. Sprague and Colodey (1989) reviewed and summarized the research in the following way. Untreated kraft mill effluents should not cause overt sublethal effects in aquatic organisms at concentrations of about 1/20 of the LC50, which would usually be in the vicinity of 1% or 2% effluent. Well-treated kraft mill effluent is only mildly toxic. It would not be expected to cause sublethal effects at concentrations below 1%, and sometimes effects are documented only at concentrations above 10%. Some of the most meaningful work has been done in artificial streams by the National Council of the Paper Industry for Air and Stream Improvement (NCASI); they found favourable communities of organisms, including spawning fish of several species, in well-treated effluent at concentrations of 9% and higher.

Some of the recent physiological studies of fish downstream of Canadian mills were reviewed by Jones et al. (1991), but the findings did not add greatly to knowledge of the actual concentrations of effluent that were causing effects. Recent work indicates that the induction of mixed-function oxidases in fish, and reduction in sex steroids, may be the most sensitive indicators of exposure to pulp mill effluent (information from researchers summarized in Sprague 1992). Much more information on toxicity can be expected over the next few years, as the result of major projects by Canadian researchers.

2.2.2 *Toxic chemical components*

Summary. Existing synopses of data on toxicity of chemical components of BPME are currently being amalgamated and updated by Environment Canada. An earlier review had indicated that not enough information was available for developing new water quality guidelines for important chemicals in BPME.

Compiling information on toxicity of chemicals in pulp mill effluent is an ongoing activity. Dr. John Leach of B.C. Research in Vancouver provided a base of information on toxicity of individual chemical components of BPME (Leach 1991). Of about 160 chemicals screened, 30 were of primary interest for toxicity and received intensive searches, but it was concluded that a lack of information precluded development of new water quality guidelines for the substances. Suntio et al. (1988) provided a useful list of chemical characteristics, toxicities, and amounts in the waste streams of mills, and N. Henderson of B.C. Ministry of the Environment has also compiled toxicity data on key components of BKME. These data are currently being combined and updated for a comprehensive toxicity database on key single chemicals in BPME (S. Walker, Environment Canada, pers. comm.). The tables of toxicity data will be used to determine whether sufficient toxicity data now exist to derive single-chemical guidelines for the major compounds found in BPME.

2.2.3 *Major substances contributing to toxicity of kraft mill effluents*

Summary. Major causes of acute toxicity in BPME are (a) resin acids and fatty acids, (b) chlorinated resin and fatty acids (c) chlorinated phenolics, and (d) a variety of neutral substances such as alcohols and ketones. Resin acids contribute a major proportion of acute lethality, but decompose readily, as do fatty acids. About ten chlorinated phenolics, resin acids, and fatty acids contribute appreciably to acute lethality; these low-molecular-weight substances show toxicity, bioaccumulation and persistence that are only moderate in degree.

The main toxic substances are commonly reported as resin acids and fatty acids, diterpene alcohols and ketones, pitch dispersants, and a range of chlorinated substances including lignins, phenolics, and chlorinated resin and fatty acids (see below and Table D.1). Acute lethality of the more important toxicants occurs mostly in the range of one-half to a few mg/L (Fig. 1).

The main **resin acids** contributing toxicity are dehydroabietic, abietic, isopimaric, and pimaric, occurring as acids and soaps. The fatty acids are lesser contributors, and the main ones are palmitoleic, oleic, and linoleic. The life of most resin and fatty acids in surface waters is a few days; the most persistent appears to be dehydroabietic acid with a half-life of about 6 weeks. Sublethally, resin acids affect the functioning of blood in fish, as well as respiratory organs, enzymes, liver and particularly the management of energy exchanges. The Ontario Ministry of Environment recommends pH-dependent water quality guidelines, with 0.025 mg/L of total resin acids at neutral pH (Taylor et al. 1988).

Toxic **neutral substances** include alcohols (pimarol, isopimarol, abietol, dehydroabietol), aldehydes, and ketones. They may contribute up to 30% of the toxicity of BPME.

Most of the organochlorines in BPME (about 80% of AOX [1]) are high-molecular-weight (MW >1,000 units) chlorinated lignins which may persist for decades but are not very toxic.

Chlorinated substances of low molecular weight, consisting of a handful of chlorinated phenolics, resin acids, and fatty acids, account for much of the acute lethality. They are lethal to fish at about 1 mg/L or a few mg/L. They are moderately bioaccumulative, but are readily excreted by fish in clean water. They are only moderately persistent, with half-lives of a week, or a few weeks.

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[1] AOX signifies Adsorbable Organic Halogens. It is a measure of the amount of halogen in a sample that is bound to organic matter. For pulp mill waste, essentially all of the halogen will be chlorine. AOX is measured by adsorbing the organic matter in a sample onto activated carbon, purging non-organic halogen, burning the carbon and passing the gas from that action through a micro-coulometric titration to measure the amount of halogen.

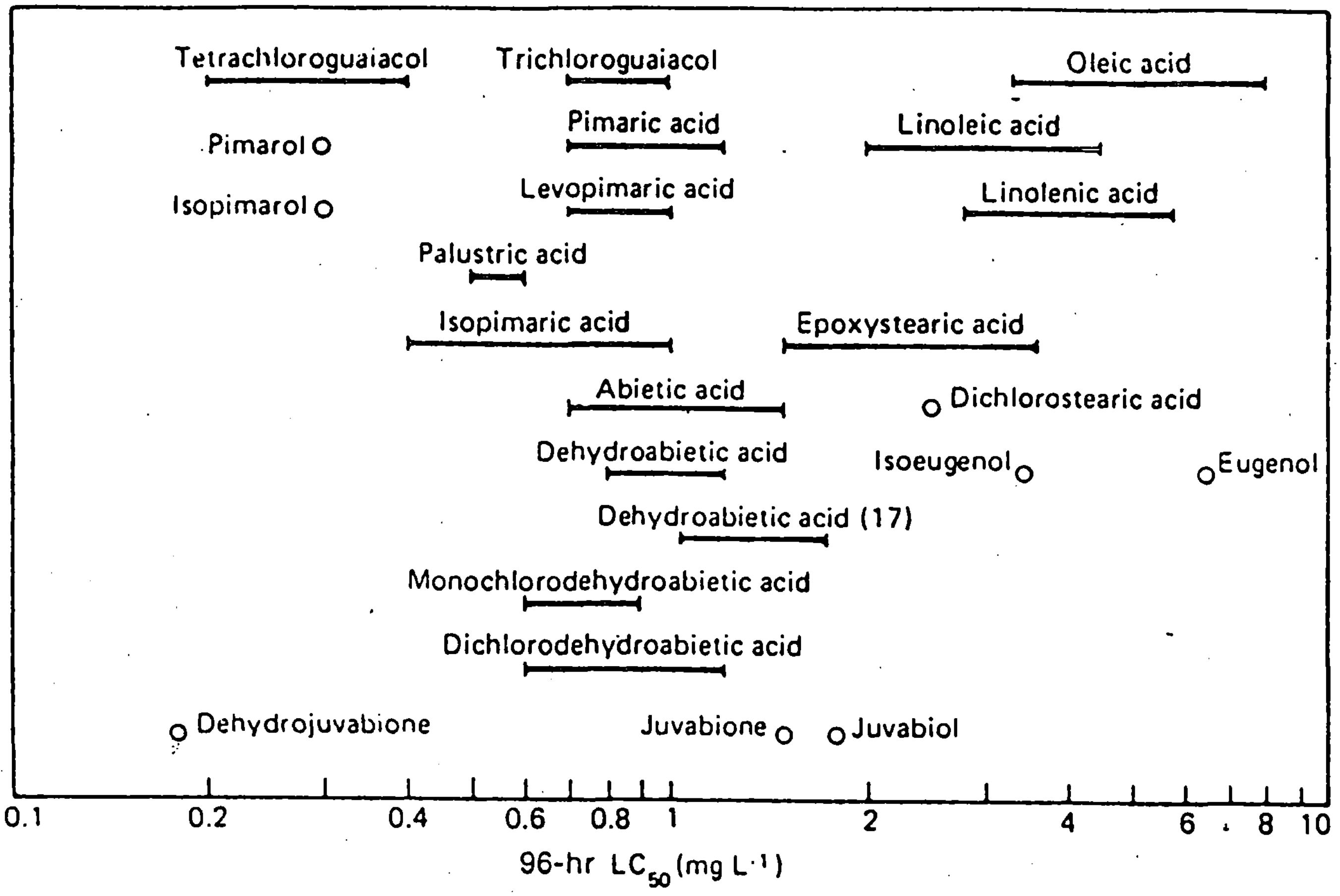

Fig. 1. General ranges of lethal concentrations for fish, of common toxicants in pulp mill effluent, as summarized by Chung et al. (1979).

The four major categories of chlorinated phenolics of toxicological interest are phenols, catechols (2-hydroxyphenols), guaiacols (2-methoxyphenols), and vanillins (2-methoxy-4-formyl-phenols) (Fig. D.1). The individual chlorinated substances that are most common and considered to contribute most of the acute toxicity are:

- the chlorinated resin and fatty acids monochloro- and dichloro-dehydroabietic, dichlorostearic, epoxystearic;
- the chlorolignins (phenolics) 4,5-dichlorocatechol, 3,4,5-trichlorocatechol, tetrachlorocatechol, and 2,6-dichlorohydroquinone; and
- the chlorinated phenolics 2,4-dichlorophenol, 2,4,6-trichlorophenol, and di-, tri-, tetrachloroguaiacol.

2.2.4 *Recent confirmation of chemicals present*

Summary. Recent detailed monitoring at nine Ontario kraft mills shows that average concentrations of the known toxic substances conformed to historic data, showing that the earlier work retained its validity. The sum of resin acids in the average Ontario effluent would appear to be lethal to fish, as would the sum of chlorinated resin acids and phenolics.

The monitoring of nine Ontario kraft mill effluents under the MISA program produced a great deal of chemical data. Comparison with earlier measurements from other places indicates that the major chemicals described in section 2.2.3 continue to be present at similar concentrations (comparison in section D.7). Many of the new and old values fit very closely. Conclusions on toxicity of BPME drawn from accumulated information in the literature should be relevant for Canadian effluents discharged in 1990-91.

Chemical measurements on these recent Ontario effluents showed that the major toxic substances were at concentrations which could cause the effluents to be lethal. The simple sum of average concentrations for 6 resin acids was 1.25 mg/L, which is into the lethal range. The sum of 9 chlorinated phenols was 6 mg/L, above the range of 1 to 2 mg/L for known LC50s of individual chlorophenols.

2.3 Guidelines for individual chemicals and categories

Summary and recommendations. Canadian guidelines exist for categories of chlorinated phenols that are important in BPME. Guidelines by category of resin and fatty acids could be developed from existing information. That would achieve general guidelines (by class) for the main toxic components of BPME.
*Lack of toxicity data precludes development of guidelines for important **individual** chemicals. A program could also be mounted to test individual chemicals and supply the required toxicity data. Effort might also be devoted to developing and testing models to describe effluent toxicity in terms of the joint action of multi-chemical mixtures.*

From section 2.2, it appears that the major chemicals in BPME causing acute toxicity are known. The development and use of water quality guidelines would be useful for these chemicals. If the current compilation by Environment Canada confirms a continuing lack of information for individual substances (Leach 1991), guidelines set by category of chemical compound could be appropriate.

For **chlorinated phenols**, Canadian guidelines already exist, not for individual substances but by category according to degree of chlorination (CCREM 1987). A more recent assessment of relative toxicity of classes of chlorinated phenolics (Kovacs et al. 1991) shows agreement with the Canadian guidelines. The remaining major group of toxicants consists of the **resin and fatty acids**. It might be feasible to develop class guidelines for those substances, judging by the rationale produced by the Ontario Ministry of the Environment (Taylor et al. 1988).

Alternatively or additionally, a testing program might be sponsored to amass the requisite toxicity data for water quality guidelines for the most important chemicals in BPME. The sublethal tests for 30 substances might be accomplished with about half a million dollars (section D.8).

While guidelines for single chemicals and classes of chemicals have innate validity, when a mixture of toxicants is present it is not sufficient to simply apply individual guidelines, one by one. There is abundant evidence that joint toxic action will take place among the chemicals (Appendix G). Some system of integrating toxic effects of all the individual chemicals would have to be adopted. That field of joint sublethal toxicity still requires both theoretical and practical study to develop and validate methods. Any resources available would be well placed if they went to **develop a standard model for assessing mixture toxicity in BPME**, at the sublethal level. In particular there is need to know more about joint action of substances in different toxicological categories (different modes of action, see sections 3.3.2 and 3.3.3).

2.3.1 Potential usefulness

The advantages of guidelines for single chemical, guidelines by class of chemical, and water quality objectives developed from the guidelines, include the following.

- They follow a standard, proven and familiar approach.
- Compliance with objectives may be assessed with reasonable precision using chemical measurement. Chemical measurement of a few substances can be more economical than biological measurements such as toxicity tests or survey.
- Knowing which particular chemical or chemicals is of concern allows for efficient planning and design of control measures, both before and after a discharge commences. Waste treatment systems are more easily designed since treatability data are available for many substances.
- Modelling of fate in the environment must be done on a single-chemical basis.

Some potential disadvantages include the following.

- Chemical guidelines give only predictions of harmful and safe concentrations, and could be in error in any given situation. In particular, bioavailability of a substance may not be evaluated by chemical analysis
- The potential for joint action of different toxicants is not considered and could lead to under-protective situations.
- All toxicants in a discharge may not be known, and that is the case for BPME.
- There may be insufficient toxicity information to develop defensible guidelines for most of the 30 to 50 main toxicants in BPME.
- For a complex discharge such as BPME, chemical methods of checking for compliance of a large number of individual substances could mean a relatively expensive monitoring program.

It is recommended that single-chemical guidelines continue to find major use in relation to discharges of pulp mill effluents. There is obvious relevance and importance of some individual substances or conditions in the receiving water, for example particular resin acids, chlorinated phenols, ammonia, and dissolved oxygen. Certain guidelines deserve reconsideration (section 2.1). Single-chemical guidelines could be developed for those compounds for which sufficient data exist.

2.4 Persistent bioaccumulative substances

A guideline based on body burden of each persistent bioaccumulative toxicant is a desirable single-chemical approach for any lipophilic toxicant which tends to partition into ecosystem compartments other than water. Environment Canada is currently developing for CCME, a method for deriving tissue residue guidelines for protecting wildlife which consume aquatic organisms. Persistent bioaccumulative toxicants do not seem to be present in BPME, except for chlorinated dioxins and furans, but if it became evident that they were present, tissue residue guidelines should be applied.

2.4.1 Rationale for guidelines based on body burdens

> ***Summary and recommendations.*** *Toxic lipophilic substances may be well assessed by body burdens in aquatic organisms, but inaccurately measured by water sampling. This approach can provide guidelines for aquatic organisms, and some were listed in the U.S.A. two decades ago for pesticides and PCBs. Environment Canada is currently developing a method for deriving tissue residue guidelines for the protection of wildlife which consume aquatic life.*

Hydrophobic substances may be on or in particles, sediments or organisms, but may not travel as molecules dissolved in water. Measurements in water samples may be difficult, unrepresentative, or show major fluctuations.

In human health, it is the practice to protect human consumers by using body burden limits for lipophilic substances that accumulate in freshwater or marine food organisms. As is well known, areas on the B.C. coast have had fisheries closed because of pollutional status for chlorinated dioxins and furans, judged by measurements of body burden instead of by water samples.

Limits for body burdens of mercury and PCBs are well known, and they also exist for some pesticides. Tissue residue concentrations were recommended as guidelines of environmental quality some time ago in the U.S.A. (NAS/NAE 1974).

2.4.2 Relevance of body burden limits for pulp mill effluent

Summary. *Aside from chlorinated dioxins and furans, there is little evidence of dangerous persistent bioaccumulative substances in BPME. There is little information for the major components of BPME, relating body burdens to toxic effects. For these reasons it does not appear useful, at the present time, to develop guidelines based on body burden, specifically for components of BPME.*

An up-to-date review of toxic substances in BPME, including bioaccumulation, has been carried out by Environment Canada (Jones et al. 1991). Apparently there are relatively few strong persistent bioaccumulative substances in BPME. Aquatic animals accumulate the more highly chlorinated phenols, guaiacols and catechols, and also tetrachloroveratrole, a transformation product of chloroguaiacol. Continued exposure in a given location leads to appreciable body burdens in aquatic organisms, but if fish move to clean water, most organochlorines are eliminated in days or weeks.

The literature fails to provide direct studies oriented to pulp mill effluents, of links between bioaccumulation and degree of harmful effect on fish and aquatic wildlife [2]. However, there have been general studies of non-polar narcosis-causing chemicals which are relevant to BPME; a threshold of sublethal toxic effect seems to be associated with a total concentration within the animal of 2×10^{-5} moles/kg (all such toxicants summed, see section J.3). Those narcotic substances are excreted fairly readily from aquatic organisms, and are not the persistent bioaccumulative substances of interest here.

Regulatory limits for human food are sometimes applied for presumed protection of predators in aquatic food webs, an approach that might occasionally be appropriate. However, a very high percentage of the diet of some wildlife predators may be composed of aquatic organisms, and tissue residue levels more restrictive than those recommended to protect humans would often be appropriate. If bioaccumulative organochlorines or other substances were identified in BPME and associated with harmful effects, guidelines could be developed for body burdens.

A major project by the British Columbia Ministry of Environment deals with human health guidelines for particular components of BPME in fish, as discussed in sections 5.2 and K.4.

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[2] There has been an appreciable amount of work attempting to associate body burdens of chlorinated dioxins and furans with effects on wildlife, especially aquatic birds. Those families of organochlorines were specifically excluded from the terms of reference of this report.

2.4.3 Potential usefulness

The advantages of tissue residue guidelines, and environmental quality objectives developed from the guidelines, include the following.

- Some toxic substances travel through ecosystems via sediment, organic matter, or living organisms and are virtually undetectable in the surface waters themselves.
- Residues in food organisms can be the most important source of some toxicants, for susceptible predators.
- Some of the most hazardous substances are lipophilic, accumulate in organisms, and are best assessed as tissue residues.
- The technique represents a direct measurement of toxicant in organisms, and reduces any error in predicting from ambient concentrations.
- Monitoring for adherence to environmental quality objectives for tissue residues is conveniently done, since the sampled organisms have integrated over time, any fluctuating levels of exposure.
- Monitoring for adherence to residue objectives is a convenient management tool to attempt to avoid problems of bioaccumulation in an aquatic food-web [3].

Some potential disadvantages include the following.

- For pulp and paper effluents in surface waters, it appears that there is not a major problem of persistent bioaccumulative toxicants, aside from chlorinated dioxins and furans.
- Unlike a water quality guideline, a tissue residue guideline is of limited predictive use, for example in deciding in advance of a planned discharge, on the maximum amounts of a substance that could be safely discharged (tactic no. 2 of a general strategy). It is generally too complex a situation to predict from amount discharged, to the concentrations in resident organisms. Modelling could assist prediction but is not yet definitive.
- Further knowledge is required for some substances, relating body burdens to degree of harmful effect in the organism or its predators.
- Measuring tissue residues of individual substances does not in itself predict the joint effects of two or more substances present simultaneously.

It is recommended, in general, that tissue residue guidelines should be developed for appropriate substances. This is a potentially strong tool for interpreting environmental quality, and very important problems with persistent bioaccumulative substances exist. At the same time, this technique does not seem to be of great importance for evaluating BPME in surface waters, for substances other than chlorinated dioxins and furans.

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[3] This is not, strictly speaking, monitoring of "environmental effects" (tactic no. 3 of the strategy outlined in Appendix B) since the presence of excess tissue residues is not in itself a biological effect but is predictive of an effect. If the tissue residue guidelines and objectives have been well designed, then survey results that show residue levels within the objectives should mean that biological effects have been prevented in the aquatic ecosystem, which would be the desired goal of a management strategy.

3 Chemical systems for predicting toxicity of mixtures

> *Existing water quality criteria have been derived for single toxicants, yet it is rare to find natural waters in which only single toxicants are present.*
>
> Robert L Spehar and James T. Flandt (1986)

A number of approaches have been used to predict toxicity of mixtures from chemical measurements. The simplest approach might adopt some chemical measurement that would include a "class" of similar substances, and assume that the class measurement would give some overall assessment of toxicity. The AOX approach with pulp mill effluents was somewhat similar to that and is described in section 3.1.

More detailed approaches have measured (chemically) the individual components of a mixture, converted that to expressions of the individual toxicities, and used a model to attempt to predict the overall toxicity. Two such methods that have been used (Toxicity Equivalents and toxic units) are described in sections 3.2 and 3.3. The two methods use essentially the same simple model under different names, and are described separately here for convenience.

Predicting the toxicity of a mixture of substances in receiving water, from chemical concentrations of individual substances, is in keeping with the approach of water quality guidelines and objectives. It is a predictive tool, and fits tactic no. 2 of a control strategy (Appendix B) in the same way that water quality objectives fit. The use of a model for combined toxicity does, however, add a further "layer" of prediction onto the basic approach of water quality guidelines or objectives, and therefore increases the possibility of inaccurate prediction.

3.1 AOX as an unsuitable "class characterization" of toxic effects

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Summary. Adsorbable organic halogen measured in bleached pulp mill effluents is not well correlated with toxic effects of the effluents, particularly at recent lower levels of chlorination in pulp mills.

The toxicity associated with a reduction in adsorbable organic halogens (AOX) decreases much more precipitously than does the AOX itself, when less chlorine is used to bleach. Canadian workers have estimated that as AOX decreased from 5.7 kg/t to 3 kg/t (less than a 2-fold drop), the total toxic effect of organochlorines in the effluent dropped from 100 to about 18 units (more than a 5-fold drop). Another recent summary showed that a 3-fold decrease in AOX was associated with an approximate 50-fold drop in total organochlorine toxicity (see Fig. F.2).

The organochlorines of most concern, the chlorinated dioxins, appear to show a decline that is even steeper than those mentioned above, as AOX decreases.

Recent surveys downstream of Ontario mills indicate that all mills, not just those using chlorine, stimulate the suite of biochemical symptoms in fish that were reported in Swedish studies of the 1980s (increase in detoxification enzymes of the liver, reduction in sex steroids, etc.). This is additional evidence that organochlorines are not alone in contributing toxicity to mill effluent.

Small amounts of evidence suggest that the low-molecular-weight fractions of AOX ("fractionated AOX") do not show an improved correlation with toxicity.

3.2 Toxicity Equivalents

Summary. The presumed joint effect of several toxicants belonging to the same chemical family may be expressed as a single number by using factors based on toxicity, relative to the most toxic substance in the family. The "TEQ" system has been accepted by the scientific world for chlorinated dioxins and furans. A similar system is available for the chlorinated phenolics in pulp mill effluent. The TEQ system is essentially the same as the toxic units model discussed in section 3.3.1.

For chemically similar toxic chemicals, the method of *Toxic Equivalents (TEQ)* may be used to simplify prediction of their joint effect. The most toxic chemical is generally adopted as the standard for the group. Each other chemical is given a *toxicity factor* according to its degree of toxicity compared to the standard chemical. The amount (or concentration) of each substance is expressed in terms of the *equivalent* amount of the standard substance. The equivalent amounts (TEQs) for all substances are added together to give a single value that predicts overall toxicity, and is easy to work with. The method is, in fact, essentially the same as the toxic units method (section 3.3.1), but is often perceived as a separate method and is conveniently discussed in this separate section.

The TEQ method has received general acceptance by scientific and regulatory communities for **mixtures of chlorinated dioxins and furans.** There are 210 different polychlorinated dibenzo-p-dioxins *(PCDDs)* and polychlorinated dibenzofurans *(PCDFs)*, with a similar basic structure of two benzene rings, but differing in number and position of attached chlorine atoms. The most toxic member of these families is 2,3,7,8-tetrachlorodibenzo-p-dioxin *(2378-TCDD)* and other isomers are expressed as approximate TEQs of that substance, by applying appropriate factors.

There is now general recognition of a standard international set of TEQ numbers for TCDDs and TCDFs (NATO 1988). *Toxicity factors* from 1.0 (2378-TCDD itself) to 0.001 were selected by groups of experts, and published by NATO, for 11 substances or similarly-chlorinated groups of substances (Table F.1). To use the method, the weight (or concentration) of a given substance is multiplied by its toxicity factor, thus expressing it in terms of an amount of 2378-TCDD that would have equal toxic effect. All such values for individual components of the mixture are added together to yield the total Toxicity EQuivalent or TEQ of 2378-TCDD.

3.2.1 *TEQ systems for chlorinated phenolics in pulp mill effluent*

Summary. A recent TEQ system for chlorinated phenolics in BPME is based on sublethal toxicity to aquatic animals. Most of the toxicity factors agree with those for chlorinated phenols as used in existing Canadian water quality guidelines. Other Canadian work has set up TEQ systems within three groups of substances: chlorinated and unchlorinated phenolics; resin acids; and fatty acids. Agreement was good to fair, between predicted and observed sublethal toxicity of effluents to Ceriodaphnia, when contributions from the three categories were summed.

Toxicity factors were assigned to **chlorinated phenolics in BPME** by Kovacs et al. (1991). The four major classes of phenolics were designated as phenols, catechols, guaiacols and vanillins. Selected literature for sublethal effects was used to assign toxicity factors, with a major assumption that substances from the four classes that had the same degree of chlorination would have the same degree of toxicity. Pentachlorophenol was taken as the base of the system, with the other toxicity factors ranging downwards two orders of magnitude (see Table 1). General patterns of relationships and separate information supported the assumptions and the factors calculated by Kovacs et al. (1991).

Table 1. Toxicity factors for chlorinated phenols, catechols, guaiacols and vanillins as estimated by Kovacs et al. (1991). A comparison is given with toxicity factors derived* from the Canadian water quality guidelines for chlorinated phenols (CCREM 1987).

Degree of chlorination of phenolic substances	Toxicity factor of Kovacs et al.	Guidelines of CCREM	
		Guideline μg/L	= tox. factor*
Pentachloro-	1.0	0.5	1.0
Tetrachloro-	0.48	1.0	0.5
Trichloro-	0.23	18.	0.028
Dichloro-	0.11	0.2	0.4
Monochloro-	0.05	7.	0.1

* The toxicity factor is obtained by dividing the guideline for a given category by the guideline for pentachlorophenol which is taken as the standard. For example tetrachlorophenols are about half as toxic as pentachlorophenol and receive a factor of 0.5.

The new toxicity factors estimated by Kovacs et al. (1991) show general agreement with those already published in the **Canadian water quality guidelines** (CCREM 1987). The Canadian guidelines assign concentrations (µg/L) according to the degree of chlorination of five categories of phenols, and Kovacs et al. do the same. The relative toxicity ratings from the two sources agree exactly for one of the categories, and have differences of 2-fold to 8-fold for the other categories (Table 1).

An examination of the reasons for the larger differences might be in order, but the general agreement must be considered good.

The TEQ approach was carried further by Holloran et al. (in press), as part of a useful exercise in planning a mill in Alberta that would meet environmental requirements. They summed toxicity within each of three classes of chemicals, **phenolics, resin acids and fatty acids**, but did not add toxicity between classes. Preliminary toxicity factors of Kovacs et al. (1991) were assigned to all groups of chlorinated phenolics. They added certain unchlorinated substances (**phenol, cresol, guaiacol, catechol and veratrol**) to the category, assigning factors of 0.001. Factors ranging generally from 0.2 to 0.75 were assigned to **9 resin acids** and **10 fatty acids**, based on values of 1.0 for the most toxic ones, isopimaric acid and chlorostearic acid.

The system of adding toxicities within the three groups (categories) of substances achieved predictions of toxic effects that seemed reasonable (Holloran et al. in press). Certainly there was practical benefit in being able to summarize lists of chemicals measured in pilot-plant effluents, with single predicted values for toxicity. This is another indication that **reasonable predictions of sublethal effects are obtained by adding contributions for different categories of toxicants.**

Using the data of Holloran et al. (in press) for five effluents, I added together the predicted toxicities of all three categories of substances, and then compared with actual sublethal tests done on the effluents (see section F.2). The best agreement was shown by one effluent for which the actual toxicity was 1.1 times higher than predicted, in other words almost exact agreement. The worst agreement was an actual toxicity that was 3.0 times higher than predicted, a correlation that might be considered fair, considering the exploratory nature of the project.

Among people interviewed, there was general approval of the toxicity factors for chlorinated phenolics, and for the principle of adding their toxicities. Most of those who commented on the TEQ approach suggested caution about extending the system to other categories, because of the current lack of knowledge about toxicity of specific substances, or about what substances are present in BPME (section F.2.1).

3.3 General models for toxicity of mixtures

> *... no-effect levels of separate chemicals may have little meaning for mixtures. ... It can be concluded from published work that, for most complex organic mixtures, the joint acute action of toxicants is either strictly additive or slightly less than strictly additive and that antagonistic or more than additive effects are not prevalent.*
> *In mixtures of chemicals that act through similar and different modes of action, ... sublethal joint toxic action generally has been found to be less extensive than at lethal levels, but to range between concentration additive to partially additive.*
>
> Steven J. Broderius (1991)

> *We suspect that, in the case of pulp and paper effluents, the toxic effect may be controlled by **the sum of the effects** of perhaps 50 chemicals.* [emphasis added]
>
> Suntio et al. (1988)

Research on toxicity of aquatic mixtures continues to receive little emphasis, but recent years have brought some findings that are important for developing and using water quality guidelines. Some of the findings may be listed as follows, and they are explained more fully in the subsequent text of this section.

- Toxic action may be contributed to a mixture, by individual substances in that mixture that are only at **1/17 of their individually effective concentration.**
- **Near-additivity** of toxic contributions by individual substances is being found frequently.
- These results are being found for **sublethal** toxic action, not just lethality.
- Additive or near-additive effects now seem to be almost standard for toxicants which act by the same physiological mechanism. Furthermore, there are some cases of appreciable joint action by substances in different chemical categories, which have clearly **different mechanisms of toxicity** within the organism.
- The above items signify that for mixtures of pollutants, there is **no such thing as a firm threshold of toxicity,** below which a substance is harmless.

In the real world, there are often two or more toxicants present at once in a given habitat, and that is likely for surface waters affected by BPME. In addition to single-chemical water quality guidelines, it would be appropriate to search for other techniques which would predict the results of combined action. Models for evaluating mixture effects exist, and there are precedents in other fields for using them.

3.3.1 *Models, terminology, and toxic units as a simple approach*

Summary. Some classical models of joint action appear to require knowledge of the mode(s) of action, before classifying and naming the type of joint toxicity. An empirical system of nomenclature is recommended, which starts with the expectation that joint actions of different toxicants will sum together. The model accordingly describes mixture toxicity by one of the terms: supra-additive; additive; infra-additive; no addition; or antagonism.
The toxic units model may be used to assess or predict the toxicity of a mixture of chemicals. The concentration of each chemical is expressed as a proportion of its threshold concentration for causing a specified sublethal effect, when it is acting individually. (Alternatively, concentration could be expressed as a proportion of its water quality guideline). The proportions for the individual chemicals toxicants are added together. The assumption is made, that a sum of 1.0 would be at the threshold of effect by joint action, and sums >1.0 would cause the effect.

There is diverse terminology for the joint action of substances acting simultaneously on organisms. Some terms are used in different ways by various people and groups. Classical terminology may be difficult to comprehend or be fashioned in terms of internal mechanisms of action, which may not be known in aquatic toxicology. Some systems of terminology are described in sections G.1 and G.2, but the following system is recommended.

Terminology can be explained by examples using two chemicals, "Toxicant A" and "Toxicant B". Each has an effective concentration, causing an effect (let us say failure to reproduce), and that concentration can be represented as 1.0 without specifying the units (i.e. 1.0 Toxicant A causes the effect separately, and so does 1.0 Toxicant B separately). Concentrations below the effective level may be described as proportions, e.g. 0.5 Toxicant A (see Fig. 2).

To actually decide on the type of joint action, according to the following examples, one would have to do toxicity tests combining various strengths of the two chemicals, and see if the effect was achieved, or not, as the result of joint action. However, the following examples cover the different types of action, and it may help to visualize them if the values are located on Fig. 2.

Additive If two substances act simultaneously and their effects sum together as if they were the same substance, then the joint action is simple addition.
This is often called "concentration addition" which implies that the toxicants have the same mode of action.
E.g. 0.5 Toxicant A + 0.5 Toxicant B = 1.0 effect (i.e. the effect is just caused)
0.1 Toxicant A + 0.9 Toxicant B = 1.0 effect

Supra-additive Two substances produce an effect greater than would be expected from simple addition of their individual contributions towards the effect.
Also called "more-than-additive" or one type of "potentiation".
E.g. 0.5 Toxicant A + 0.2 Toxicant B = 1.0 effect

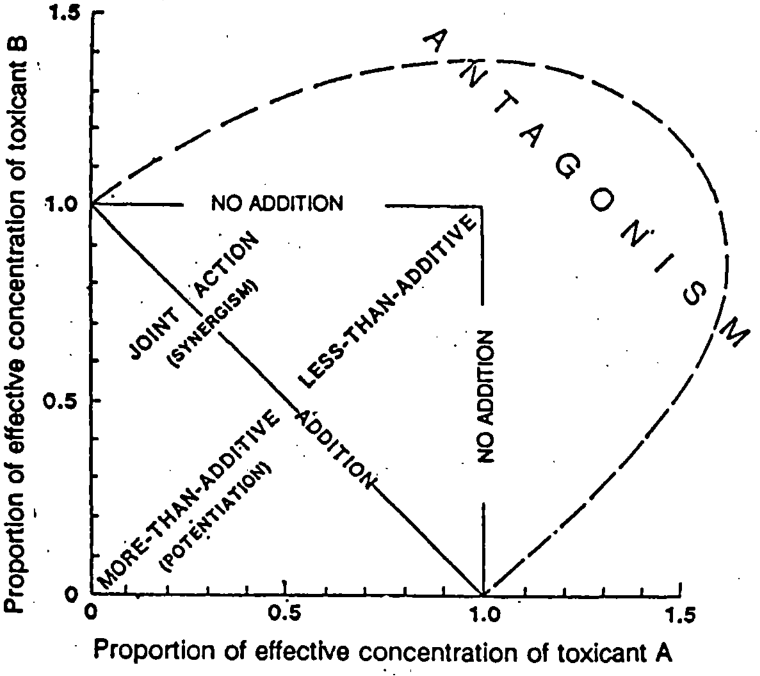

Fig. 2. Terminology that may be associated with various kinds of joint action of two toxicants acting simultaneously. After Sprague (1970).

Infra-additive Two substances co-operate to some extent in causing an effect, but not in a fully additive way.
Also called less-than-additive.
E.g. 0.8 Toxicant A + 0.7 Toxicant B = 1.0 effect (just)

All three categories above represent "joint toxicity", i.e. the two toxicants work together in some degree to cause an effect that neither could cause by itself at its concentration.

No addition Each toxicant operates independently as if the other were not present, and does not influence the action of the other.
This could be considered a special case of infra-addition. Some authors call this one case of "response addition".
E.g. 0.3 Toxicant A + 1.0 Toxicant B = 1.0 effect (just)

Antagonism The presence of one toxicant reduces the effect of the other. The power to diminish or counteract could be reciprocal or only one way.
Often misused as if it meant infra-additive.
E.g. 0.6 Toxicant A + 1.0 Toxicant B = 0.5 (no effect or partial effect)

The **toxic units** model or approach has been used in various scientific fields. To use the method, the concentration of each toxicant is not expressed in chemical units, but as a fraction of its threshold of sublethal effect (or of its water quality objective, or guideline), i.e. in *sublethal toxic units (SLTU)*. This would use the following formula.

$$SLTU = \frac{C_A}{TEC_A}$$

where SLTU = Sublethal Toxic Units
C_A = Concentration of Toxicant A that is actually present in the water
TEC_A = Threshold Effect Concentration of Toxicant A (or No-Observed-Effect Concentration, or Water Quality Guideline for Toxicant A)

The concentration must be in the same units for the top and bottom of the fraction (e.g. mg/L). The effect could be any defined sublethal effect. For a mixture of many toxicants, the formula would simply be an extension of the one above. The proportion would be calculated for each toxicant in the mixture, and all of those values would be added together.

$$SLTU = \frac{C_A}{TEC_A} + \frac{C_B}{TEC_B} + \frac{C_C}{TEC_C} + \frac{C_D}{TEC_D} \quad etc.$$

The model could be used in a **predictive mode, following the primary function of water quality guidelines**. If the sum of the individual components equalled 1.0 or greater, the designated effect would be expected. Or if individual water quality guidelines were used as the denominators, a total ≥ 1.0 would be interpreted to indicate that the mixture guideline was exceeded (i.e. the water would not be "safe"). The method is likely to be over-protective since joint actions tend to be additive to less-than-additive, rather than more-than-additive.

As a simple example, let us say that the sublethal thresholds of toxicity for substances A, B, C and D, as known from the literature, are 100, 200, 400, and 800 µg/L. Let us say that the four substances are expected in the effluent from a mill, and after dilution into the receiving water, maximum seasonal concentrations are expected to be, respectively, 4, 6, 10 and 20 µg/L. Substituting in the above formula, the four fractions are 0.04, 0.03, 0.025 and 0.025 as dimensionless values. The sum is 0.12 sublethal toxic units, and no effect would be expected on this basis, since the value is less than unity. However, if water quality guidelines had been developed for the substances A to D, no doubt safety factors of 10 would have been applied to the sublethal thresholds (or LOELs) to allow for more sensitive species. The guidelines would have been 10, 20, 40, and 80. Substituting the guideline values as the denominators and the expected concentrations as the numerators in the equation, the sum of the fractions would be 1.2. That total would not meet the guideline mixture if it were set up to assume simple additivity. If a similar water quality objective had been adopted for the locality, the prediction would be that the effluent would not meet the objective. Redesign of the industrial process or waste treatment would be in order, to achieve lower discharge levels.

The model could also be used **to interpret the manner of joint action** of the component chemicals in the mixture. This would be done in a similar way to the definitions and explanations given above for two toxicants (A and B).

Other models for joint toxicity are reviewed in section G.4. Some are less useful for guidelines because they have drawbacks such as limitation to lethality or binary mixtures.

3.3.2 Evidence of additive lethality

Summary. There are now many studies showing additivity or near-additivity of lethal effects in diverse mixtures. Much research deals with pairs of toxicants but some with up to 50 chemicals together. Contributions to joint toxicity have apparently been shown by toxicants present at only 1/50 and 1/400 of their individual LC50s. The mixtures include organic chemicals with insecticides, polluted river waters, oil drilling fluids, municipal effluents, pulp mill effluents from debarking, kraft pulping, caustic extraction and mechanical pulping. For 130 samples of North American pulp mill effluents, the additive LC50 predicted from chemical analysis was within 30% of the actual LC50 in 73% of the cases.

Work in the Netherlands showed that each of 50 similar-acting organic chemicals, each present at only **2% of its own lethal concentration, contributed fully** to lethality of the mixture. More Dutch work with mixtures of another 50 organic chemicals showed that concentrations as low as 1/400 of an individual LC50 appeared to contribute to the joint lethal toxicity. Other tests showed near-additivity for lethal action to guppies, for mixtures of 8 to 24 organic chemicals which had several different actions and included pesticides. Concentrations of a chemical as low as 1/10 of its individual lethal level contributed to the joint action.

A project of the U.S. EPA assessed 175 pairs of toxicants, and most of them showed additive or somewhat infra-additive toxicity (Fig. G.1). Only a few showed a "complex type of joint action" or "a markedly more-than-strictly-additive type joint action" (Broderius 1991). Other European and Canadian evidence of near-additive lethality of mixtures is in section G.3.2.

In the 1970s, researchers at B.C. Research accurately predicted toxicity to coho salmon by chemically measuring the components of pulp mill effluent, then summing them by toxic units. None of eleven effluents from debarking, kraft pulping, caustic extraction and mechanical pulping, gave clear evidence of departing from simple additivity of component toxicity. Measured toxicity averaged 1.03 times that predicted from chemical analysis (range of values = 0.81 to 1.54), within the normal range of experimental error for toxicity and chemical measurements (Leach and Thakore 1977).

An even larger study by B.C. Research assessed 130 samples of effluents from various mills (Leach and Chung 1981). Nineteen important toxicants were measured chemically, and summed by toxic units to yield a predicted LC50. For 73% of the samples (shown in Fig. 3) the predicted LC50 was within 30% of the actual measured LC50. For the most part, the effluents were slightly more toxic than predicted, but in general there was good agreement. This impressive work from B.C. Research does not appear to have been further developed.

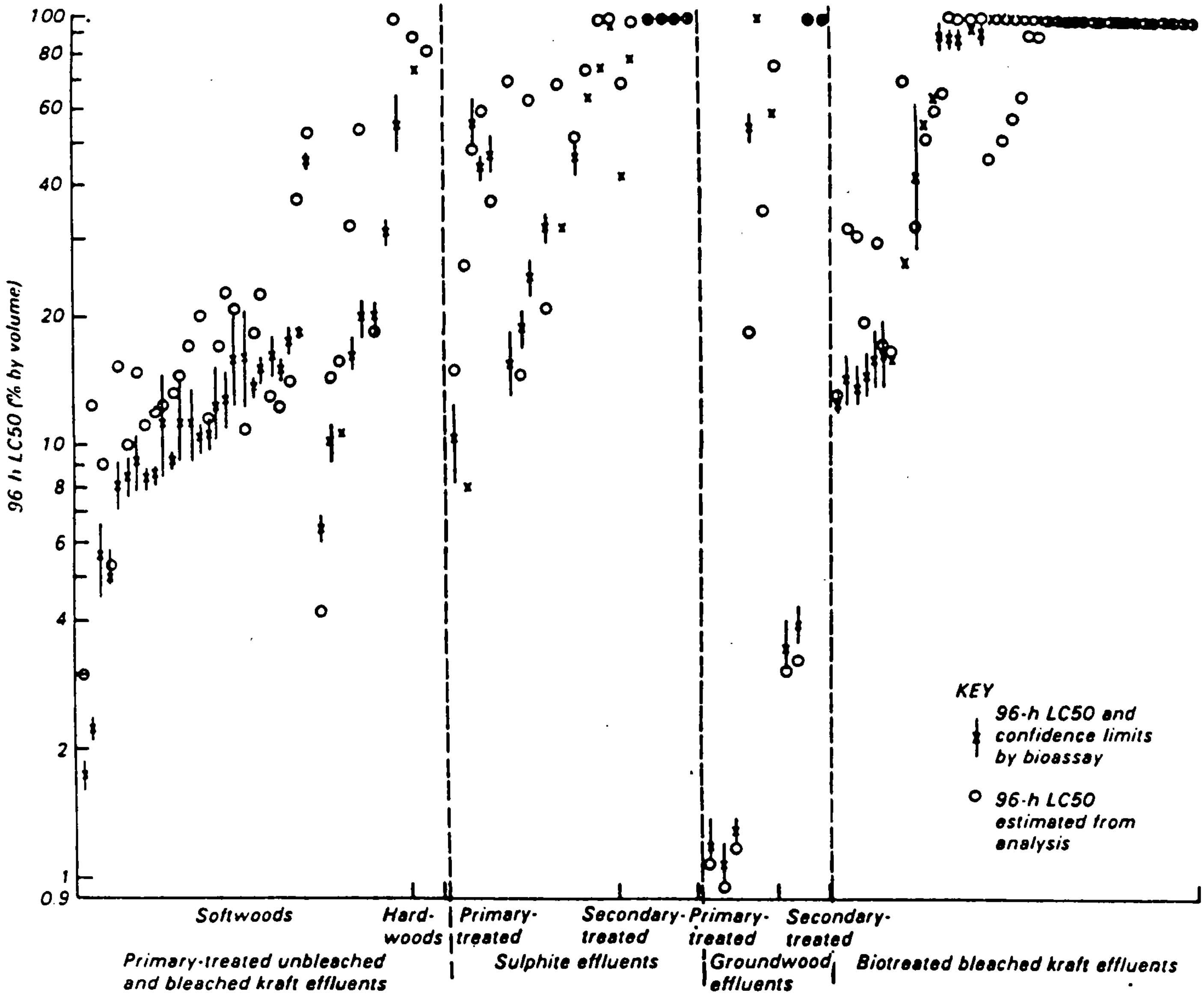

Fig. 3. Comparison of lethal concentrations of pulp mill effluents measured for trout, with the LC50s predicted by the toxic unit method from chemical analyses of nineteen important components of effluents. From Leach and Chung (1981).

3.3.3 *Sublethal toxicity of mixtures*

Summary. There are a few sublethal tests on mixtures. They indicate that there may be no sublethally 'safe' concentration of a chemical when it is part of a mixture of toxicants in the receiving water. Tests of 5 to 25 substances indicated that mixtures of similar-acting toxicants contributed at least half of their individual sublethal effect to the overall toxicity of the mixture. A mixture with several modes of action contributed about half of individual toxicities to the action of the mixture.

For water quality guidelines, toxicity at the sublethal level is of greatest importance, since we should not assume that the lethal tests described above will predict joint sublethal actions. Sublethal tests are few, but indicate that joint action does take place. The rapid sublethal tests that are now available make it easier to explore sublethal toxicity of mixtures, and it is to be hoped that more research results will be seen.

The results of Wong et al. (1978) are widely known in Canada. The photosynthetic rate of algae dropped by 70% when exposed to a mixture of 10 metals, each at its own water quality objective as set for the Great Lakes. Photosynthesis was reduced by 40%, even when each metal was at one-tenth of its objective. Various factors may have contributed to these results, including early objectives that were set too high on an individual basis, and combined action.

Similar findings were obtained by Dutch workers for mixtures of 5 to 25 organic substances having similar actions. Toxic effect on reproduction of *Daphnia* was assessed by the toxic units method. Mixtures were effective at 1.5 to 2 SLTU, i.e., the **individual substances retained one-half to two-thirds of their potencies in the mixtures.** Individual substances were at concentrations as low as 1/17 of their individual effective concentrations.

Sublethal additivity was nearly perfect, for effects on Daphnia reproduction caused by ordinary organic chemicals (Narcosis I type), in mixtures of 10 and 25 chemicals; some concentrations were only 4% of the individually effective ones. Even for a mixture of 14 substances embracing several types of chemical structures and modes of action, Daphnia reproduction was affected at 1.9 SLTU. In other words each of the **dissimilar toxicants apparently contributed about half of its toxicity to the action of the mixture**, when each was present at about 1/7 of its individual effective concentration.

The work cited in this sub-section suggests that there may be **no such thing as a sublethally "safe" level** (NOEC) of an individual substance when it is part of a mixture of substances with similar action, i.e. even very low concentrations may contribute some toxicity to a mixture. Some other experiments in the literature show a variety of results including lack of combined action, and some of these are mentioned in section G.5. Yet some of the findings above indicate that a single-toxicant approach to assessing water quality may not predict the effect of complex mixtures on aquatic ecosystems. In particular, it has been estimated that there may be 30 to 50 important toxic chemicals in pulp mill effluent, and those numbers are of the same magnitude as used in research that showed near-additive sublethal toxicity.

3.3.4 Guidance from other experts and other fields

Summary. Most of the experts interviewed did not offer guidance on mixture toxicity. Two major reviews in aquatic toxicology concluded that simple addition and the toxic units model were suitable for general use for applied problems. Certain other disciplines had major interests in joint toxicity, but little to offer in the way of tools to handle multiple mixtures. Many industrial hygiene agencies in the western world use the toxic unit model to set limits for mixed airborne pollutants of similar action.

Of the **people interviewed** for this project, most did not offer advice on developing guidelines for mixtures of pollutants. One expert suggested caution because of the lack of field validation and another was in general agreement with recent research findings.

Two major reviews of mixtures in aquatic toxicology were published in 1991. In view of the sophistication of Christensen and Chen (1991) as statisticians and modellers, and the long-continued study of mixtures by Broderius (1991), it is of some significance that they concluded that a simple *toxic unit* model is sufficiently accurate in most circumstances to be used for prediction and in fact for regulating discharges.

Broderius (1991), in the quotation at the head of this section, may be paraphrased as saying that mixtures of organic toxicants show a joint lethal action that is additive or almost additive. At the sublethal level, whether the chemicals in a mixture have the same mode of action or different modes, they tend to show weaker joint action than in lethal tests. The sublethal joint action has been found to range between addition and partial addition (i.e. infra-addition).

"In the absence of calibration data, we believe that the water quality criterion, ... based on concentration addition, is appropriate. It is more protective for aquatic life against adverse effects from chemicals in a mixture than individual limits for toxicants based on no addition, and because near-concentration addition is so common, it is also more generally applicable" (Christensen and Chen 1991). [Here, "concentration addition" signifies the toxic units model.]

Aquatic management agencies may now be starting to deal with mixture toxicity. In Europe, EIFAC (1987) proposed that the joint effects at higher toxicity levels (lethality) should be assessed by assuming additive action for both similar and diverse toxicants. EIFAC recommendations are very tentative, however, for dealing with sublethal mixtures.

Some other disciplines have strong interests in mixture toxicity and their work has been reviewed in hope of uncovering useful techniques (Parrott and Sprague in press). Pest control workers, and horticulturists concerned with air pollution, did not have superior techniques for evaluating thresholds of effect for multi-chemical mixtures. Some methods could only be applied to pairs of toxicants, and many used subjective interpretation. Some mathematicians have written extensively on the subject, but in an academic fashion assuming knowledge of modes of action. The conclusion that other fields of endeavour cannot offer advanced techniques for practical applications, is supported in a review by Foulon (no date).

The field of occupational hygiene is an applied field which has been forced to provide guidelines for mixtures of toxic substances, especially for airborne contaminants in the work-place. From an extensive analysis, Foulon (no date) summarizes: "A review of the approaches taken by most regulatory agencies indicates that some form of additive model is generally applied to the problem of mixtures." This is true for substances having similar mode of action, but for unlike actions, the hygienists assume independent action and depend on single-chemical guidelines. Among the agencies using a simple toxic units approach are the American Conference of Governmental Industrial Hygienists, United Kingdom Chemical Industries Association, Netherlands Government Institute for Public Health and Environmental Protection, a (former) U.S.S.R. health agency, and the World Health Organization. More complex approaches emphasizing case-by-case risk assessment are used for hazardous waste sites by the Canadian Contaminated Sites Remediation Program, and by the U.S. EPA.

From this review, it seemed that aquatic toxicologists were as advanced as any group, or more advanced, in discovering patterns of simultaneous sublethal action. Other disciplines had developed tools for relatively narrow topics. Industrial hygienists had been the most progressive in applying a model for joint toxicity to guidelines and regulatory procedures.

3.4 Potential usefulness

The advantages of guidelines for mixtures of substances, based on single chemicals, and of water quality objectives developed from the guidelines, include the following.

- All known toxicants in a discharge such as BPME can be included in the system, with more likelihood of assessing the total effect, than would be the case for assessing substances individually.
- The approach is predictive and could be used in planning before a discharge takes place.
- There is some scientific evidence that toxicants with similar action are additive in their effects when present in mixtures. There is some evidence that components of BPME are approximately additive in their effects which therefore can be predicted.

Some potential disadvantages include the following.

- Any system of predicting joint toxicity of a mixture of substances could have major errors, especially for mixtures of unlike substances.
- No single system of assessing joint toxicity has been universally accepted, although use of the TEQ/toxic units model is not uncommon.
- For a complex mixture such as BPME, extensive, repeated and expensive chemical measurements would be required for monitoring of compliance.
- There has been little field validation of systems to predict mixture toxicity, despite considerable laboratory testing.

It is recommended that an attempt be made to find a **general** approach for toxicant mixtures in aquatic systems, based on concentrations of individual substances, and/or classes of substances to predict effects of mixtures in receiving waters. The toxic units model (≈ TEQ model) is recommended for consideration. For the present, application to pulp mill effluent would have to be partly based on classes of chemicals.

4 Guidelines from direct sublethal testing

It is necessary to use toxicity itself as an additional control parameter.
Rebecca Hanmer (1991)

Parts per million is not a meaningful measurement of environmental health.
Murray G. Johnson, Ph.D.

The current Canadian water quality guidelines are derived in part from sublethal toxicity testing since data from such tests are used in developing the chemical guidelines. This section of the report deals with defining a procedure of direct toxicity testing as a water quality guideline. The section starts by reporting, from other countries, some recent direct uses of biology in water quality guidelines, or in related activities that are more control-oriented. Then the possibilities for toxicity testing as a guideline for effluent in surface water are examined. The section concludes that guidelines based directly on toxicity testing should be given consideration, whether or not there is parallel consideration of the chemical approaches for predicting mixture toxicity, described in the preceding section.

4.1 Biological methods of managing water quality in other countries

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Summary. *The U.S.A. and Australia have functional water quality guidelines that specify toxicity tests and an endpoint of no sublethal effect in the receiving water. Britain is planning a biotic index of community health as a quality guideline, with objectives derived for local surface waters. Swedish "characterization of industrial discharges" includes sublethal testing for certain dilutions in receiving water. There should be consideration of the direct use of sublethal endpoints in Canada, through the development of a scientific guideline for BPME that is based on toxicity testing.*

Although this report deals with scientific guidelines, some of the clearest evidence of advances is in the regulatory procedures being practised in other places. The practices may be examined for any underlying guidelines or other scientific bases which could be useful in Canada. Biological techniques from elsewhere are described in this sub-section, whether they be purely scientific or regulatory in their nature. Those of us involved in water quality work should be aware of the new developments in use of biological techniques.

Canada pioneered biological tests in regulations of 1971, with a standard requirement of a trout lethality test on pulp mill effluent and a specified result of the test. That was to be applied in each situation as a regulatory standard. Since then, the lethality test has retained its position in Canada, while some other countries have leap-frogged into use of sublethal testing. The change in approaches is appreciable. The trout lethality test on the effluent represents a "best-technology" approach (tactic no. 1 of a strategy, Appendix B), and is not a water quality objective; a requirement of non-lethality applies for any volume of discharge into any size of waterbody. Some other countries use this lethal test but may also apply a requirement for no sublethal effect in the receiving water (tactic no. 2).

In the U.S.A., sublethal toxicity tests have a requirement for a national biological monitoring program as the result of implementation of effluent testing since 1984. In practice, this program consists of specifications for short sublethal tests with a fish, an invertebrate and a plant, used in tests with effluent. At each discharge location, the controls that grow from those tests is a requirement for no sublethal effect at concentrations expected at the edge of a mixing zone in the receiving water.

Conventional (chemical) national water quality criteria are simultaneously part of the balanced U.S. approach. At the control/regulatory level, the approach uses not only chemical water quality objectives and toxicity testing, but also best-treatment requirements and ecological survey to check for satisfactory conditions, i.e. all three tactics in a desirable strategy (Appendix B).

In Britain, the use of biotic indices is an enlightening example of using biology in a quantitative way for water quality purposes. The biotic indices express the health of the aquatic invertebrate community, and the index including its methodology and scale of values, is essentially a graded national water quality guideline. The values of the index are used to set a water quality objective in a given local situation. (Of course, the biotic index is a particularly useful tool since it can also be used in a monitoring role; as an ecological technique it can check whether conditions are satisfactory in the receiving water, i.e., the monitoring of tactic no. 3, Appendix B).

The British guideline of a biotic index is not recommended for development in Canada at this time. Although biotic indices work well in Canadian rivers, they are not yet commonly used here. The example is cited because of its innovative direct use of biology as a guideline.

A Swedish handbook on water quality management shows how the results of toxicity tests, with safety factors, can be indirectly designated as water quality objectives. The statement of methods in the Swedish handbook is intended for national application, so the statement could be considered a narrative water quality guideline, although not designated as such. The handbook describes a sensible tiered approach for "Characterization of Industrial Discharges" (CID), i.e. judging their potential harmfulness. The 4 components of the approach are toxicity, degradability, bioaccumulation, and chemical nature. Toxicity is first judged by tests of acute lethality, and if receiving-water concentrations are expected to be >0.1 LC50, the discharge is considered acutely toxic and remedial actions are to be undertaken. If the expected effluent concentrations in receiving water will be >0.01 LC50, the discharge is considered "capable of causing damage", and the investigation should proceed with sublethal tests of Tier 2. The relevant point is the direct use of a toxicity end-point as an objective, and by implication, the use of a standard toxicity method as a national guideline.

4.2 Toxicity as a water quality guideline

Summary. Toxicity as a water quality guideline would be based on a sublethal test or tests done with the effluent to assess/determine the combined effects of the mixture in the receiving water. The threshold-effect concentration would be the benchmark for establishing a local water quality objective, which would be stated as a maximum concentration of effluent in the receiving water. The overall approach and its defined methodology would constitute a water quality guideline, since the same procedure would be used in all locations for estimating the maximum concentration in the receiving water.
Short, sublethal, whole-organism tests are recommended, such as life-cycle tests with daphnids and algae, and early life-stage tests with fish.

The only way to measure toxicity is with a biological test. Chemical tests can never prove or disprove the presence of toxicity, although they can explain the cause of toxicity, or predict toxicity, sometimes very accurately.

Biological tests should have the advantages of: (a) measuring effects directly; (b) measuring combined effects of several toxicants in an effluent; (c) evaluating components for which no chemical measurements were done; and (d) assessing components whose toxicity is unknown. Some potential disadvantages are listed in sections 4.7 and I.2.1.

It follows that direct measurement of toxicity is a logical feature to use as a water quality guideline. One use of guidelines is to serve as a scientific base for water quality objectives and standards for protection of aquatic organisms from harmful sublethal effects, something that is directly measured by sublethal toxicity tests.

The actual water quality guideline would be a narrative statement of a method for determining toxicity of an effluent, and of estimating a maximum concentration in the receiving water. The toxicity would be determined from sublethal tests with a series of dilutions of the effluent itself, to estimate the Threshold-Effect Concentration (TEC) for any particular effluent at any given time. The TEC with an appropriate safety factor (section 4.3) would be the maximum concentration in the receiving water[4]. The water quality guideline is thus the method of determining the maximum concentration, and that method is a single standard one, that would apply universally. When the method was used, it would produce a water-quality objective, a number which would by definition apply to a particular place (and time).

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[4] The TEC is used throughout this section as the endpoint of a sublethal test. It is the geometric mean of the No-Observed-Effect Concentration (NOEC) and the Lowest-Observed-Effect Concentration (LOEC), as recommended by Environment Canada (1992a). Other endpoints might be considered superior and could be used in a similar way, perhaps with slightly different safety factors. The NOEC is sometimes used as the endpoint, and CCREM (1987) uses the equivalent of the LOEC (the LOEL). Another endpoint of sublethal testing could be the IC25, the Inhibiting Concentration for a 25% reduction in some biological rate compared to a control.

Since effluents vary from place to place and time to time, there cannot be toxicity data that apply to all effluents from pulp mills that bleach, or even to an effluent from one mill. Therefore there cannot be a fixed percentage concentration of pulp mill effluent that would apply in all places. Data on sublethal effect must be obtained for each particular effluent at the particular time of interest. It must be tested with an agreed-upon sublethal test or battery of tests.

A legitimate initial objection to this effluent-testing approach might be that it is too variable with time and place to be the basis of a scientific guideline. That is true in terms of percentage concentration of effluent, but the remedy is to **abandon thoughts of percentage concentration as the primary measure of strength.** One should instead, think in terms of "degree of sublethal toxicity" as the measure of strength of an effluent. That toxic strength should be reduced in the receiving water to non-harmful levels. The scientist might think of the water quality guideline as a fixed fraction of the TEC (e.g., perhaps 1/10 of the TEC). The mill manager concerned with control could think of the maximum degree of toxicity acceptable in the effluent, for the particular dilution into receiving water as calculated and agreed upon for that location.

For regulatory purposes, the guideline might be used as the basis of a local water quality objective or standard. After testing, calculation could be made to determine whether or not the objective would be exceeded in the receiving water after dilution. This is essentially the procedure used by the U.S. EPA (see Appendix H).

Whole-organism sublethal tests should be used for meaningful results. Recent tests for reproduction and growth of small organisms are almost as fast as lethal tests with fish, and standard methods are being published by Environment Canada.

4.3 Safety factors

Summary and recommendations. *A safety factor in the region of 0.1 should be applied to a threshold based on sublethal tests with the effluent of concern.*
The purpose of the safety factor would be to allow for sensitive species and individuals in the receiving community. The water quality guideline would be defined by specifying the sublethal test(s) to be used, and the value of the safety factor.
The CCME currently uses a factor of 0.1 applied to the threshold for sublethal effect, and a value of that magnitude receives some support from European institutions, for managing effluent toxicity.

The threshold(s) of observed effects derived from testing an effluent may not be protective of an entire aquatic community. A safety factor would allow for more sensitive species in communities, for more sensitive individuals in a population, for effects that might occur with longer exposures than were possible in the laboratory, or for possible inaccuracy of the tests.

The CCME has defined safety factors which may be used to derive water quality guidelines from sets of data on toxicity. A safety factor of 10 was selected for use on the lowest nonlethal chronic endpoint in the available data for the toxic material. That is, "the most sensitive LOEL is multiplied by a safety factor of 0.1 to arrive at the guideline ..." (CCME 1991). Other techniques with application factors are used if it is necessary to work from acute data.

A value of 1/10 of the NOEC receives some support from two European organizations, for managing waste discharges. OECD (1987) and the Swedish Environment Protection Agency (SNV 1991) both use biological tests as **prototype water quality objectives.** Various dilution ranges are given as part of evaluation processes, and there are implications that concentrations of concern would be those that are higher than 1/10 of the NOEC.

Some perspective may be gained by considering safety factors in human toxicology, which appear to be more conservative. A safety factor of 100 is often used if there are good data for chronic exposures of animals, and that value is composed of a factor of 10 intended to protect sensitive individuals, and an extra order of magnitude to allow for differences between humans and the test species (e.g. mice).

4.4 Recommended methods for guideline

By specifying the sublethal tests to be used and the value of the safety factor, a water quality guideline would have been defined, that could be applied to any effluent.

To develop a water quality guideline for effluents that was based on sublethal testing, it would be necessary to select an appropriate safety factor. The choice should consider evidence on valid safety factors for ecological systems, but a suitable factor would almost certainly be in the general vicinity of 0.1. As mentioned above, the factor of 0.1 is the one presently used in developing guidelines for single chemicals from chronic sublethal studies, and would appear to be suitable for effluents.

The sublethal toxicity tests should be done with at least two types of organisms. The modern whole-organism tests with durations of two to seven days should be satisfactory (Environment Canada 1992a, b, d). The TECs (threshold effect concentrations) would be calculated as the geometric mean of NOEC and LOEC, and the safety factor would be applied.

Other techniques, of extrapolating from lethality results to estimate "safe" levels, are available but are not recommended here for pulp mill effluents. Use should be made instead, of the rapid sublethal tests mentioned above.

4.5 Precision and validity of a biological guideline

Summary. The accuracy of a modern aquatic sublethal toxicity test cannot be determined since there is no way of measuring toxicity, except the toxicity test itself. Precision is satisfactory, however, with coefficients of variation that are often in the range 20% to 40%, similar to the variation found for chemical tests. Validity of sublethal tests on effluents has been established; the tests correlate well with conditions found in the receiving water by survey of the biota.

Variation exists in sublethal testing, but recent comparisons show no greater inconsistency than in other kinds of measurements. Formal studies of interlab variation for tests with early life-stages of fathead minnows and other fast sublethal tests, show coefficients of variation ranging from 13% to 52% (section I.5). That range of precision is as good as, or better than, the ranges that may be reported for chemical analyses of priority pollutants. For example, a comparable average inter-laboratory coefficient of variation for chemical measurements was ≥60% (Rue et al. 1988).

Validation of the short sublethal tests, for their correlation with observed conditions in receiving waters, has been deliberately and extensively studied in the U.S.A. in recent years. The studies show reasonably good predictions from the single-species tests to the community in the receiving water. Agreement of predictions at a multiplicity of sites was found in 81%, 84%, 88% and 90% of cases (see Fig. I.6).

4.6 Expert advice

Most people interviewed were comfortable with the concept of using sublethal testing as a water quality guideline. Some gave positive support, mentioning failures of certain chemical measurements to predict toxicity.

It appears that biochemical effects within fish (MFO induction, etc.) occur at concentrations of pulp mill effluent that are below the NOEC for the usual sublethal tests with daphnids and fish larvae (work of Drs. J. Carey, K. Munkittrick, M. Servos, and their associates at the Canada Centre for Inland Waters (C.C.I.W.), Sprague 1992). At C.C.I.W., T.G. Williams is developing a 6-day test for MFO induction in the lab, for effluent testing. Most experts advised that biochemical tests were not yet ready for use in developing guidelines. The safety factor applied to the TEC of whole-organism tests might be a satisfactory solution.

4.7 Potential usefulness

The advantages of guidelines based on direct testing of toxicity, and water quality objectives developed from the guidelines, include the following.

- The overall action of all toxicants present is assessed. Substances that might be missed in chemical screening are included. Substances whose degree of toxicity is not known are included.
- The evaluation can include the relative toxicity of different forms of substances, and effects of modifying factors such as pH if they are included in the test.
- Only one parameter need be measured and specified.
- The toxicity test can be used in a predictive manner, to some extent, by testing an effluent or simulated effluent and specifying the maximum concentration in receiving water (tactic no. 2).

Some potential disadvantages include the following.

- The aquatic community in the surface water of interest may contain species that are much more sensitive than the species used for the toxicity tests (a difficulty that is not restricted to this approach).
- The substance(s) primarily responsible for any measured toxicity are not identified and, therefore, chemical methods would be needed as a follow-up to determine which substances should be controlled.
- The procedure is unlikely to detect low concentrations of persistent bioaccumulative substances that could accumulate in ecosystems over time.
- It is usually ineffective to attempt toxicity testing in receiving water for predictive purposes. Tests must be done with the effluent, and perhaps some ancillary conditions might not be exactly representative of the surface water (e.g. pH).
- Toxicity of some substances might increase after some time in the receiving water, because of release from unavailable forms or decomposition to more toxic components.
- For designing waste treatment, the parameter "toxicity" may not be very helpful, and specific chemical description may be needed.

It is recommended that the concept of toxicity as a guideline in surface waters should be actively developed. This would involve considering suitable methods of toxicity testing and suitable safety factors to extrapolate to sensitive species. A toxicity guideline should be considered whether or not it is decided to examine mixture guidelines based on chemical measurement (section 3); I consider this one of the most important items covered in this report.

5 Modelling

This final section of the report focuses on modelling techniques that could support many aspects of the work on guideline development, including those in the preceding sections. The modelling is an exercise in mathematics, of course, but the information modelled is drawn widely from chemical, physical, biological and other sources of data. The results are predictive, not empirical, and so despite the excellent progress outlined in some sub-sections below, a cautious supporting role for modelling is suggested.

5.1 Quantitative structure-activity relationships

> *From the present study it is concluded that QSARs, extrapolation, and EP [equilibrium partitioning] can be used to derive quality criteria for the aquatic environment ... [for] relatively unreactive, nonpolar, organic chemicals ... that act by narcosis ..."*
>
> Van Leeuwen et al. 1992.

Summary. QSAR predicts toxicity of substances from their chemico-physical structure. QSARs also predict bioaccumulation, degradability, and joint action of similar substances in a mixture.
The chlorinated phenolics of BPME follow standard QSARs.
QSARs could have supporting roles in several aspects of guideline development. Predicting the degrees of toxicity and bioaccumulation of various substances would be suitable uses. QSAR should be useful to assist the development of tissue residue guidelines for chemicals in BPME.

QSAR deals with relationships between the physical and chemical characteristics of a chemical and its activity in the environment, particularly toxicity. Predicting which chemicals are likely to be the most dangerous is an excellent use of QSARs. One of the major tools in this field is the relation of the octanol-water partition coefficient to many aspects of bioaccumulation and toxicity.

Research on QSAR in aquatic toxicology usually centres on four categories of toxicants, with (1) and (2) being most used.

(1) Narcosis I type, such as 1-octanol and most of the chlorinated phenolics of interest in pulp mill waste, which cause general membrane perturbation.
(2) Narcosis II, such as phenol, which acts by depolarization of membranes.
(3) Uncouplers of oxidative phosphorylation such as dinitrophenol.
(4) Inhibitors of electron transport, such as hydrogen cyanide.

The chlorinated phenols so important in BPME not only fall into category (1) of general, "narcosis-type" toxicity, they also follow the general QSAR.

5.1.1 *QSAR and guidelines for general-action toxicants in BPME*

Summary. QSAR for narcosis I chemicals allows prediction of toxic concentrations in water, sediment, and tissues, according to a recent Dutch paper. An internal concentration for fish which is judged protective, is 2 x 10 $^{-5}$ moles/kg. That finding should be relevant to tissue residue guidelines.

Since the joint toxic action of non-specific toxicants is essentially additive, the residue criterion given above applies to the total body burden from a mixture.

From molecular weight and octanol-water partition coefficient (K_{ow}), van Leeuwen et al. (1992) showed that toxicity could be predicted for narcosis I chemicals including alcohols, chlorinated benzenes and toluenes, diverse ketones, ethers, and chlorinated alkanes. The work was based on 102 organic chemicals with 19 species of aquatic microbes, algae, invertebrates and vertebrates. Prediction was claimed for toxic concentrations in water, sediment, and tissues.

The internal toxic concentration (ITC) of narcosis I substances, for fish, which was judged to protect 95% of the species in a community, was estimated as 2 x 10^{-5} moles/kg. That value for tissue concentration may be seen as a benchmark of environmental quality, and used as such for predictive matters. Since the residue level applies to the **total body burden from a mixture**, it is usefully related to mixture toxicity (section 3).

The Dutch researchers generalized that a **safety factor of 10**, below the lowest whole-organism NOEC in the laboratory, estimated the ITC.

QSARs would be beneficial in developing guidelines for general-action organic chemicals in BPME. For BPME guidelines based on individual substances, QSAR should be part of the development effort. QSAR could predict which chemicals were most toxic and worthy of attention. As the work progressed, consideration should be given to our present knowledge, and whether it is sufficient to base a guideline on multi-chemical summation of tissue residues. This approach of total tissue residues for narcosis-type chemicals of BPME could be one of a set of "overlapping" guidelines [6].

(Although QSARs are often directly related to ease of uptake and bioaccumulation, this section does not deal with the same topic as section 2.4 which is concerned with persistent bioaccumulative toxicants. The toxicants of concern in that other section are strongly bioaccumulative substances which stay in the body of an organism for a long time, in other words they are not metabolized but build up gradually in tissues and tend to stay there. The present category of narcotic organic chemicals need not be persistent in the tissues and most

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[6] Suggested so far for consideration and possible use are: classical single-chemical guidelines; residues of single persistent bioaccumulative substances; mixture toxicity following the toxic units model; direct sublethal testing with a safety factor; and this one, residues of narcotic-type chemicals, probably considering total residue (mixtures) as well as single-chemical residues.

of those in BPME would not be. However, continual exposure of organisms could lead to deleterious effects in the long term as well as the short term. In particular, the shared, non-specific mode of action must be allowed for when considering potential effects.)

5.1.2 Expert advice

Some scientists working in this field suggested caution in using QSARs for developing water quality guidelines (see section J.4). There was support for predicting which toxicants were most important, but warnings about carrying the process too far. For example predictions to a real ecosystem would be complicated by other processes such as decomposition which affected the concentration of toxicant.

5.2 Modelling in the receiving environment

Summary. Modelling is a predictive tool for integrating mixture toxicity, bioaccumulation and QSAR, particularly if it works with dose of toxicant within organisms rather than concentrations in the water. Modelling has had good successes in predicting and interpreting chlorinated dioxins from pulp mills, and British Columbia has a major project to model human health connotations from toxicant loading from mills.

Despite the above, a supporting role was evident in developing water quality guidelines for BPME, rather than a dominant role. Specialists should be consulted on the beneficial use of modelling.

Mathematical modelling is a way of understanding how toxicant residues in organisms relate to water quality guidelines. Accordingly, modelling is relevant to several topics in this report, especially Toxicity Equivalents, Mixtures, QSAR, and Bioaccumulative Substances. This is a special field which is very fast-moving in areas such as pharmacodynamics. Environment Canada would be well-served to get specific advice from a qualified expert, on how it might use modelling to unify its approach to the topics listed two sentences above. Specific models are required for the kinds of toxicants in BPME, since they do not fit the pattern of hydrophobic substances (e.g. PCB) commonly used in the literature.

The relevance of modelling to pulp mill discharges is indicated by the conference on exactly that topic held in December, 1991, at the University of Toronto Pulp and Paper Centre.

The British Columbia Ministry of Environment is actively engaged in modelling as a bridge between residues in fish and the discharge loading (considered fully in section K.4). At present the project in B.C. is not developing water quality guidelines but is (a) a regulatory exercise, (b) concerned with human health rather than the ecosystem, and (c) quite site-specific in nature. The project aims to produce objectives for specific substances or fractions of AOX.

Chlorinated dioxins and furans in fish, as the result of mill discharges, have been modelled with outstanding success by Muir et al. (in press). These substances are not included in the present report, but the example provides encouragement that similar things could be done for other components.

Internal dose of toxicant is related to many of the topics discussed in this report, but has broad importance as a primary basis of modelling. The need for, and advantages of, dose-related approaches for pulp mill effluent has recently been described by McCarty (1991b). Other experts supported McCarty's outlook. In particular, he has shown how modelling can quantify the relationships among acute/chronic toxicity, bioconcentration/toxicity, and structure/effects.

5.3 Potential usefulness

The advantages of modelling in the development of guidelines include the following.

- Modelling based on QSARs can predict bioaccumulation, degradability, and joint action of similar substances present simultaneously in surface waters.
- Significant toxic substances and important hazards may be identified in advance by this relatively rapid and inexpensive means.
- The chlorinated phenolics which are significant toxicants in BPME, follow standard QSARs.

Some potential disadvantages include the following.

- Mathematical modelling always entails the possibility that some important aspect has been left out of the model (e.g. decomposition rate of the substance of interest). Therefore a prediction from a model to a real ecosystem must always be regarded as simply a prediction.
- A model must be for a single substance since specific numerical inputs are required.
- Progress requires people with specialized knowledge and skills in mathematics and chemical toxicology.

It is recommended that modelling including QSARs should be used to support several aspects of guideline development. Predictions of degrees of toxicity and environmental fate are of general usefulness, particularly when examining categories of substances without current guidelines. Prediction of bioaccumulation is relevant to developing tissue residue guidelines.

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APPENDIX A
LIST OF PEOPLE CONTACTED FOR THIS PROJECT

The following people were contacted and interviewed in person or by telephone for this report. The primary purposes were to learn of new techniques for establishing guidelines for effluents, and new knowledge about bleached pulp mill effluent. Information was also sought on new strategies of pollution control that included items derived from water quality guidelines.

People suggested by Environment Canada were contacted. Beyond that, there was no attempt to contact every expert, but to communicate with a broad enough network to learn of any new approaches, and get a sense of general opinions. European contacts were made, hoping to take advantage of any innovative or beneficial approaches from there. The chief impression was much stronger use of biological survey and biological criteria in Europe, compared to Canada. Some obvious sources of information, not included in the list of contacts, are United States, Scandinavia, and Australasia, because of some familiarity with approaches used in those places.

A report with complete notes on interviews (Sprague 1992) is summarized immediately below.

Summary

These interviews produced no magic answers to the questions of how to develop guidelines for effluent. The survey was broad enough to call attention to any new or innovative approaches. Many people were perplexed by the idea of water quality guidelines for an effluent, and people often talked in terms of regulatory procedures for pollution control rather than scientific guidelines. It appears that we must build upon a number of existing techniques.

Integrative biological approaches are superseding purely chemical ones in some parts of the world, and for some purposes. The United Kingdom is now setting water quality objectives for sections of river on the basis of numerical indices of the quality of invertebrate communities.

There was majority support for using sublethal toxicity tests as a base for guidelines. Useful information was obtained on fast-moving research on biochemical symptoms in fish that result from bleached pulp mill effluent (BPME) and in fact from non-bleached effluent. Biochemical effects are very sensitive, and are coming close to categorization as ecologically meaningful.

There was very limited support for various theoretical or semi-theoretical approaches with a chemical basis, such as using quantitative structure-activity relationships (QSAR) as a predictive approach. Frequent advice for caution was encountered.

A majority of people did not have a problem with the concept of using mathematical modelling to bridge parts of a process setting guidelines, and thought the modelling would gain general acceptance. British Columbia has started an interesting program to work from residues of toxicants in fish, through a site-specific fate model, to the allowable loading in effluent.

List of people

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APPENDIX B
GUIDELINES CONSIDERED IN THE CONTEXT OF A DESIRABLE STRATEGY FOR POLLUTION CONTROL

Summary. When involved in any aspect of water quality work, it is beneficial to keep in mind the overall structure of an effective pollution control program, its component techniques and their differing objectives. A good control strategy should use three separate tactics, one of which should be derived from water quality guidelines: (1) best technology, to reduce the amounts discharged; (2) water quality limits; and (3) monitoring of the aquatic community to verify that controls are working. The recent Canadian pulp and paper effluent regulations use the first and third tactics.

Those of us involved in technical or scientific work on water quality problems, even peripherally, should attempt to maintain (1) a general understanding of the main approaches/techniques available for controlling water pollution, and (2) a grasp of the objective that goes with each technique. From four decades of involvement in anti-pollution efforts, the author of this report concludes that the least successful programs have resulted from failure to consider the different objectives that may exist, the tactics and methods that can be used, and especially, **which technical methods go with which tactic and objective**. The following strategy with its three tactics, although a simplification, is a useful framework for thought. Further rationale is given in Sprague (1990b) and Sprague et al. (1991). The philosophy of the "triad" approach in the U.S.A. is similar, in categorizing techniques according to their objectives (U.S. EPA 1991), but the U.S. triad includes only two of the three tactics outlined here (one of the present tactics is split by U.S. EPA to make a triad).

(1) **"Best-available-technology" (BAT) limits** on amounts of pollution discharged.

For a pulp mill, this requires good industrial practice within the mill and/or good waste treatment. Dangerous, persistent, bioaccumulative toxicants should always be controlled by this tactic, since a limit on the amount discharged is the important thing for protecting ecosystems from such substances.

For more ordinary pollutants, BAT limits are only a starting-point since this tactic ignores the characteristics of the receiving body of water, such as dilution available.

(2) **Water-quality limits** in the receiving water. These can be derived from guidelines.

These are essential in any pollution-control scheme, since it is usually the concentration of a toxicant in the receiving water that is the important thing for the community of living organisms.

Water quality limits are the strong predictive tool that is available for use "before the event", i.e. at the planning stage for any discharge that is contemplated. Having set a water quality objective, and knowing the mixing and dilution available, calculations can be made about acceptable amounts for discharge, and industrial design can be done at the planning stage to meet the objectives.

(3) **Ecological surveys** to check that control is satisfactory. If not, tighter limits would be needed under (1) or (2) or both.

This is obviously an "after-the-event" technique, which is why it should be devoted to verifying the effectiveness of the previous two control tactics, which can be "before-the-event" or during the planning stage. Assessment of the resident biota is necessary to document an effect or lack of effect.

Although chemical conditions may be checked under in surface waters and interpretations made (e.g. do the chemical concentrations meet water quality objectives?), such interpretations remain predictions, as to whether or not an effect would be expected. The final answer must be in the biology.

The three tactics are quite different in their specific objectives. Because of that, any given chemical and biological technique is likely to be very useful for one tactic, and of no use or limited use for others. Appropriate techniques must be selected so that the objective of each of the three tactics is met.

The recent Canadian pulp and paper effluent regulations have limits for discharge of oxygen-demanding matter and suspended solids, fitting into tactic no. 1. Under the same BAT tactic, the regulations require that the effluent should be non-lethal to rainbow trout, a legitimate goal under tactic no. 1. The regulations do not have water quality limitations, i.e. tactic no. 2 is not used. *Environmental Effects Monitoring* (EEM) is part of the regulations and involves study of resident organisms, fitting tactic no. 3.

Water quality guidelines are the scientific basis for any objectives or standards under tactic no. 2. Their role in specifying concentrations of given substances is an obvious one, of primary importance in developing any control program. The tool currently uses chemical measurements (CCREM 1987), and is based on known toxicological relationships.

Certain biological techniques can be used directly in a pollution control strategy. Some form of biological survey must be used for tactic no. 3, as mentioned, to determine if there has been an effect. Biological methods are also used as part of tactic no. 1, illustrated by the existing test for acute lethality of the effluent, found in the pulp mill regulations. Finally, sublethal tests can be part of the second tactic, by using absence of sublethal toxicity as a water quality limit (see section 4).

APPENDIX C
EXISTING WATER QUALITY GUIDELINES, RELEVANT TO BPME

Table of contents

Summary. The existing Canadian guidelines for conditions in the receiving water should continue to be used to set site-specific objectives for waters affected by pulp mill discharges. There are a dozen guidelines for general conditions and specific toxic substances that are very relevant to pulp mill effluents. The existing guidelines for oxygen, pH and total phenols should be revised.

Some of the already-existing Canadian water quality guidelines (CCREM 1987) are quite applicable to bleached pulp mill effluents (BPME). Some general conditions such as reduction in dissolved oxygen are covered by guidelines that are valid for any cause of reduction, whether it be BPME or some other pollutant. Some of the specific toxic compounds in BPME already have individual water quality guidelines.

These existing guidelines should continue to be used. Where the guidelines have been used to develop objectives for bodies of water, **individual objectives should be met** in the waters receiving pulp mill waste.

However, that would not necessarily be sufficient to protect the waters, and that is why approaches are being examined for developing water quality guidelines for effluents in surface waters. Part of the problem is that BPME contains substances for which no guidelines exist. The present bank of toxicity data may not be sufficient to develop defensible guidelines for most of the substances, as revealed in an earlier study (Leach 1991, see also section D.2). The other part of the problem is that effects of the whole effluent might be appreciable, even though separate components met their guidelines. This topic of mixture toxicity is discussed in Appendices F and G.

The substances mentioned in this appendix already have Canadian guidelines, with one exception. They are listed here with comments, and some of the rationale for the comments is given in Sprague et al. (1991).

C.1 General water quality conditions

C.1.1 Total suspended solids

In past decades, some pulp mills discharged disastrous amounts of suspended solids, chiefly fibre, that had severe effects on the communities downstream. Such discharges are no longer a problem at Canadian mill locations (e.g. OMOE 1991). The Canadian guidelines for suspended solids (CCREM 1987) are strict but rational. They represent a limitation on change from the natural condition in a given waterbody, as is appropriate for a variable that is both a natural quality of water and a potential kind of pollution.

C.1.2 Dissolved oxygen

Oxygen has always been one of the problems in Canadian waters receiving pulp mill waste, because of the high loading of biochemical oxygen demand (BOD). BOD of effluents is a measure of the potential for deoxygenating surface waters, but it is the actual dissolved oxygen (DO) that is of concern to aquatic animals, and DO itself provides the water quality guidelines.

The CCREM (1987) guidelines should be revised. They provide a single concentration for warm-water biota, and another concentration for cold-water biota. Single higher numbers are also given for "early life stages", apparently of fish but not other types of organisms.

A conceptual difficulty is that any reduction of oxygen from 100.0% saturation, imposes a metabolic load on animals, at least in theory. An analogy with the oxygen needs of people may help explain. If a person started at sea level and climbed a mountain, the lowering of oxygen pressure would require progressively more effort in breathing, starting when the person left sea level, even if the effect was not noticeable at first. At about 5,500 metres elevation when oxygen pressure was halved, the effect would indeed be evident, because heavy breathing would be required to simply lie still. Aircraft pilots' manuals warn of fatigue at 3,000 m (oxygen must be carried in an airplane for sustained travel at that height); there is further warning of lassitude and cyanosis at 4300 m and loss of consciousness at 5500 m. For the mountain-climber, however, deleterious effects would be continuously graded from the start of the climb.

Applying this to water quality guidelines, there is a difficulty in attempting to set a single number for what is a graded and continuous effect. Some approaches attempt to resolve this difficulty by linking degrees of impairment to specified reductions in oxygen [7]. The CCREM document cites some of these "levels of protection" from U.S. EPA in its Table 3-7. Davis (1975) used the same approach with particular reference to Canadian species, and established three defined "degrees of effect" in a system of limits, one set of which was shown in Table 3-9 of CCREM. Complete sets of values for two types of biological communities may be compared in Table C.1.

Table C.1. Minimum levels of dissolved oxygen proposed by Davis (1975) for three "protection levels" for fish. Values are shown for two types of freshwater fish community, and Davis had four more types of communities. The temperatures are defined as "seasonal temperature maxima".

Fish community	Protection level	Oxygen, % saturation at specified temperature					
		0°	5°	10°	15°	20°	25°
Mixed fish community with salmonids	A	69	70	70	71	79	87
	B	54	54	54	57	54	63
	C	38	38	38	38	39	39
Mixed fish community with no salmonids	A	60	60	60	60	60	66
	B	47	47	47	47	47	48
	C	35	35	35	35	35	36

Since the apparent toxicity of pulp mill effluent is increased with lowered oxygen, any effluent guidelines that are developed should be suitable for the guideline level of oxygen, or else the DO guidelines should allow for such interactions. Oxygen concentrations that allowed good scope for activity of aquatic organisms would be particularly important in any waterbody receiving pulp mill effluent.

Reconsidering dissolved oxygen guidelines would be technically challenging, considering the mandate of CCME. Some expert groups have held the view that "*any* reduction of dissolved oxygen can reduce the efficiency of oxygen uptake by aquatic animals and hence reduce their ability to meet demands of their environment" and "it appears equally true for sensitive

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[7] If scientific and technical people attempt to set a single value for a substance or condition which has continuously graded effects, like reduced oxygen, we are attempting, in part, to make a social decision on "how much effect on aquatic organisms is to be allowed". An alternate role would be to provide the technical information associating the various decreases in oxygen with clearly-labelled degrees of effect (or with lack of effect). The scientific people could then offer society a choice, since decisions in any particular situation might involve factors outside the realms of expertise of the scientists.

invertebrates as for fish that *any* reduction of dissolved O_2 may have deleterious effects on their production" (NAS/NAE 1974). The Canadian guidelines have a declared goal of protecting all forms of aquatic life and all life stages during indefinite exposure. A technical solution to this would be a requirement for no reduction in dissolved oxygen. NAS/NAE (1974) did, in fact, recommend that for "nearly maximal protection of aquatic life", dissolved oxygen "should not be less than the estimated natural seasonal minimum concentration ... of that body of water for the same season". Whether that requirement remains scientifically defensible might be worth examination.

It is recommended that the dissolved oxygen guidelines be reviewed, with particular consideration of the system developed by Davis (1975) as a basis of new guidelines.

C.1.3 Hydrogen-ion concentration

While effluents of pulp mills can have extreme pH, this has not tended to be a problem in the receiving waters in recent years. If there is secondary treatment of effluent, then pH must be ecologically favourable in that treatment system and hence in the receiving water.

Since pH is another natural quality of water, here again it is challenging to design completely appropriate guidelines. The pH will have an optimum value or range for a given community of organisms, and as pH moves away from the optimum there will be graded effects that increase in severity. The conceptual problem is that different organisms and different communities will have different optima for pH. Acidophilic organisms will not do well in alkaline water, and vice versa. That creates a difficulty in assigning a single range of pH that will meet the CCREM objective of selecting guidelines that are suitable for all species.

As with suspended solids and dissolved oxygen, one response to the graded effects is to select guidelines that consist of a series of "levels of protection". That was the approach of EIFAC (Alabaster and Lloyd 1980), and it was also recommended to the U.S. EPA at one time (NAS/NAE 1974) but was not adopted. The approach is not suitable for the goals that have been adopted by the CCME. Given those goals, a concept that holds more promise for pH and other natural qualities of water is specifying the **degree of change from the natural level** or natural range.

CCREM (1987) reviews the above topics in some detail and ends with a single guideline that is a range of pH. This guideline deserves review with particular consideration of concepts for degree of change from natural.

C.1.4 Nutrients

Eutrophication of fresh water is almost always governed by amount of phosphorous because it is in shortest supply among the necessary nutrients. The effluents of most mills are low in phosphorus, generally ≤0.5 mg/L, corresponding to state–of–the–art goals for municipal treatment plants. Secondary treatment at mills would not greatly reduce the discharge of phosphorus. *Loading* of phosphorus is more important to the receiving water than is concentration in effluent, but loading from pulp mill effluents is also relatively low (Sprague et al. 1991, OMOE 1991).

Thus, nutrients are not the primary concern in discharge of BPME but enrichment can be of importance in some locations. The CCME is currently developing environmental quality guidelines for nutrients.

C.2 Chlorine and chlorinated organic substances

C.2.1 Chlorine

The Canadian guideline is carefully documented. Although much chlorine is still being used in pulp mills that bleach, one would not expect a high concentration of free chlorine in receiving waters. Available chlorine would react with other materials in the effluent.

C.2.2 Chlorinated phenols

Canadian guidelines for chlorinated phenols are arranged by degree of chlorination, based upon a perceptive analysis of the available data. The guidelines for five classes of chlorinated phenols are suitable for use in relation to pulp mill discharges to surface waters. A recent assignment of TEQ values to chlorinated phenolics (see Appendix F) is in exact to fair agreement with the Canadian guidelines.

C.3 Non-chlorinated phenols

Ordinary phenols and cresols are common in BPME in low concentrations, but they are not important contributors to toxicity (Table D.1 in Appendix D). These substances tend to be higher in the waste from corrugated and board mills but are usually within guidelines in the receiving waters.

The Canadian guidelines for chlorinated phenols are realistic and well supported (see above), but the guideline for total phenols (monohydric and dihydric) is accompanied by an acknowledgment that more specific guidelines are needed. That is true, and the existing one should be reviewed. Some priority should be given to the review since phenols downstream of a few Canadian mills have caused concern when compared with the value of the guideline.

Values above 1 μg/L, the existing guideline for total phenol, do not necessarily represent a pollution problem, because of a flawed rationale in the original development, based on protection against tainting of fish, and the rationale was incorrect. The American Fisheries Society (Thurston et al. 1979) pointed out that while 1 μg/L might be justified to "protect against tainting by chlorophenols, the criterion is not justified for ... non-chlorinated phenols. And, this stringent criterion is not justified for any phenolic for the purpose of protecting aquatic biota". The most recent guideline from U.S. EPA (the "gold book", U.S. EPA 1986b) indicates that chronic toxicity to aquatic life could occur at a concentration of 2,560 μg/L of phenol (i.e. non-chlorinated phenol) and that control of taste and odour problems in water would be achieved at an estimated level of 300 μg/L.

As recognized in the CCREM (1987) document, individual objectives should be developed for other categories of phenolics or specific phenolic substances (Thurston et al. 1979). The separate guidelines for chlorinated phenols should be used as necessary to protect against tainting, and the general phenol guideline should be revised to a more realistic level.

C.4 Metals

C.4.1 Aluminum

This metal is found in most pulp mill effluents and "alum" may be used as an additive in paper-making. However, the metal is a common constituent of the earth's surface and is present in appreciable concentrations in most surface waters. When receiving-water quality is compared with guidelines, there should be at least some consideration of the amounts *added* by a mill, beyond that already in the receiving water or in the process water. The existing CCREM guideline gives good and detailed consideration to the complicated forms of aluminum in water.

C.4.2 Cadmium, copper and zinc

The Canadian water quality guidelines for these metals are conservative. Guidelines for cadmium in water, soil, sediment and biota are currently under development as part of CEPA initiatives. Certain mills discharge appreciable amounts of one or the other of the metals, and some locations with low dilution could exceed a receiving-water objective derived from a guideline.

C.5 Other toxicants

C.5.1 Ammonia

Conservative and suitable water quality guidelines have been established for Canada. Ammonia is generally not a problem in the receiving water near mills. It is sometimes an active toxicant in regulatory tests on effluent, because it can be quite toxic at the higher pH values which may develop during the test. Such toxicity is usually more of a problem after secondary treatment, since ammonia is produced by decomposition of organic matter.

C.5.2 Other

Some other substances that have guidelines in CCREM (1987) might be considered relevant because they can be at high concentrations or high loading rates in pulp mill effluent, but they would usually be less important than the ones listed above.

APPENDIX D
TOXICOLOGICALLY SIGNIFICANT CHEMICALS IN BPME, AND SINGLE-CHEMICAL GUIDELINES

Table of contents

This appendix includes a general introduction to the substances of major concern in bleached pulp mill effluent. It is a review of existing knowledge on the topic. The appendix ends with a view of the potential for development of water quality guidelines for single chemicals or classes of chemicals that are relevant to BPME in surface waters. Chlorinated dioxins and furans are not considered here since Environment Canada is addressing them separately.

D.1 Should the focus be on untreated or biologically treated effluents?

There is a general assumption that the recent pulp and paper effluent regulations will result in secondary treatment at all Canadian pulp mills. Such treatment decreases the amounts of most toxic substances, while a few substances pass through treatment with little change.

From that, it might seem that this report should only deal with the toxic compounds in treated waste, but that approach has been rejected on two grounds. First, there are well-known cases in which secondary treatment did not function very well, so that the final effluent was more similar to untreated waste. Second, any water quality guideline must be capable of assessing all kinds of conditions in the receiving water, including unusual discharges, severe spills, or by-pass of treatment. Thus the components of untreated BPME have at least been considered in this section. If guidelines are set up for materials that are stopped by secondary treatment, then observations in the receiving water will simply indicate satisfactory conditions. If there is an unexpected problem and a by-pass of treatment, the guidelines will help to identify that problem.

This section deals only with non-volatile toxicants in BPME. Although appreciable toxicity may result from volatile substances (sulfides, mercaptans, and sulfur dioxide) those compounds are easily prevented from reaching surface waters, so they are not considered here. Similarly an unfavourable pH may account for considerable toxicity in the effluent but is also a relatively straightforward item to remedy.

D.2 Status of toxicity database

Summary. One synopsis of toxicity data on chemical components of bleached pulp mill effluent concluded that for the important chemicals without water quality guidelines, there was not enough information for developing guidelines. That information is currently being amalgamated with other databases and updated by Environment Canada, to see if guidelines can now be developed.

An information-base on toxicity of chemical components of bleached pulp mill effluent was compiled by Leach (1991). About 160 chemicals were screened for information on toxicity, and among those, 30 known to be of primary interest for toxic contribution received more intensive searches. The major conclusion was a lack of information for developing water quality guidelines for "new" substances, because of too few tests of the right kind or on a sufficient diversity of organisms.

There are other compilations, currently being combined and updated for a comprehensive toxicity database on key single chemicals in BPME (S. Walker, Environment Canada, pers. comm.). Some of the available information is tabulated by Suntio et al. (1988), and N. Henderson of B.C. Ministry of the Environment has also compiled toxicity data on key components of BKME. The base of data will be used to determine whether sufficient toxicity information now exist to derive single-chemical guidelines for major compounds found in BPME.

D.3 Major contributors of toxicity in kraft mill effluents

Summary. The usual major causes of toxicity in bleached pulp mill effluents are (a) several resin acids and a few fatty acids of lesser toxicity, (b) chlorinated resin and fatty acids (c) chlorinated phenolics, and (d) a variety of neutral substances such as alcohols and ketones.

Table D.1 generalizes on the major non-volatile toxicants. The information comes from classic findings on acute lethality by Leach and Thakore (1973, 1975, 1977), supplemented by information from McKague (1981) and Walden and Howard (1977).

The major items listed in Table D.1 are resin acids and fatty acids, diterpenes, pitch dispersants, and a range of chlorinated substances including lignins, phenolics, and chlorinated resin and fatty acids. The major categories of chemicals, which are known to contribute appreciable toxicity, are covered in summary fashion in the sections below.

Table D.1. The constituents of different streams contributing to kraft mill effluent, that are major causes of toxicity.

Effluent stream	Major toxic contributors	Lesser toxic contributors
Debarking	Resin acids Isopimaric Dehydroabietic Abietic Pimaric	Diterpene alcohols Pimarol Isopimarol Abietol Dehydroabietol
Pulping (unbleached whitewater)	Resin acid soaps Na isopimarate Na abietate Na dehydroabietate Na pimarate	Sodium salts of unsaturated fatty acids Palmitoleic Oleic Linoleic
Acid chlorination	Chlorolignins 4,5-Dichlorocatechol 3,4,5-Trichlorocatechol Tetrachlorocatechol 2,6-Dichlorohydroquinone	?
Caustic extraction	Chlorinated stearic acids Epoxystearic acid Dichlorostearic acid Chlorinated resin acids Monochlorodehydroabietic a. Dichlorodehydroabietic a. Chlorinated phenolics Tetrachloroguaiacol Trichloroguaiacol	Liquid pitch dispersants

Certain other reports and reviews could be useful for providing more details on the chemicals present in BPME, if those additional details were desired. NCASI (1989a) gives a detailed list of substances that have been found, and a detailed list of toxicities known for the various substances; from the lists it is, however, difficult to appreciate which of the substances are of most toxicological significance in an effluent. Suntio et al. (1988) also provide a detailed list, adding details of chemical characteristics, toxicities, and available estimates of amounts in the waste stream.

The acute lethality of the more important toxicants lies mostly in the range of one-half to a few mg/L. This is shown be Fig. 1 from Chung et al. (1979) which contains many of the same substances and categories listed in Table D.1. The figure has 10 resin and fatty acids, 5 chlorinated phenolics and resin acids, and 7 neutral and other substances.

D.4 Resin acids and fatty acids

Summary. Resin acids in kraft mill effluents contribute a major proportion of the acute lethality. These substances decompose readily. Fatty acids contribute a smaller amount of toxicity, and also decompose.

Resin and fatty acids, particularly the former, contribute much of the acute toxicity of kraft mill wastes. The sodium soaps of resin acids contributed 80% of the toxicity of the whitewater from kraft pulping, and sodium salts of unsaturated fatty acids contributed the rest, in a study by Leach and Thakore (1973).

In effluent from debarking of softwood, resin acids are the dominant toxic substances. Effluents from woodrooms that use wet debarking are generally quite toxic, with LC50s of 10% or lower for mills using softwood. Resin acids are found only in softwoods. Accordingly, mills debarking hardwood have great variation in toxicity of woodroom effluents, and substances other than resin acids are responsible for any toxicity present (Leach and Howard 1973, McKague and Walden 1977). Dry debarking greatly reduces toxic discharges, and today an increasing proportion of the debarking is done at sawmills, remote from the pulping operation.

In general, the lethal concentrations of resin acids and of chlorinated resin acids are close to 1 mg/L. A total of 41 estimates of lethal concentrations for fish were reported by Taylor et al. (1988), and they are all within a small range from 0.2 to 2 mg/L. These substances lose toxicity in alkaline water, and become more toxic in acid water.

At sublethal concentrations, resin acids have a whole suite of effects on fish, including functioning of blood, respiratory organs, enzymes, liver and particularly the management of energy exchanges. These effects are fully discussed in a guideline document of the Ontario Ministry of Environment (Taylor et al. 1988). That document recommends provincial water quality guidelines of 0.025 mg/L of total resin acids in water of neutral pH, but ranging from 0.009 mg/L in acidic waters (pH 6.5), to 0.06 in very alkaline waters (pH 9).

The resin and fatty acids are not persistent, and show 70% to 90% disappearance in a few days of secondary treatment (McLeay 1987). An Ontario kraft mill with an aerated lagoon showed 96% reduction of resin acids in the effluent, and 99% reduction of fatty acids (Beak 1987). Individual resin acids showed mean values ranging from 65% to 97% for percentage removal, and fatty acids showed from 71% to 100% removal (Beak 1987, Table 8.1).

The life in surface waters would usually be measured in days. The most persistent resin acid appears to be dehydroabietic acid (DHA), and it has a half-life (decomposing to half of the original concentration) in surface waters of only about 6 weeks (Taylor et al. 1988). Toxicity of resin acids may disappear in 3 to 6 days, at least under experimental conditions of decomposition (B.C. Research 1976). In a Finnish lake, within one kilometre of a kraft effluent discharge into a lake, resin acid disappearance was 97% and fatty acid disappearance was 90% (Oikari et al. 1985).

Upon chlorination, some resin and fatty acids are oxidised, while others form chlorinated compounds that are much more persistent.

D.5 Neutral extractives

Neutral diterpenes including alcohols, aldehydes, and ketones have been shown to contribute appreciable acute toxicity in BPME. Those that collected in the foam of a lagoon contributed 30% of the total toxicity, the remainder being caused by resin acids (Servizi et al. 1976). The diterpene alcohols pimarol, isopimarol, abietol and dehydroabietol were listed in Table D.1 as lesser contributors to toxicity from barking operations.

D.6 Organochlorine substances

Summary. A variety of chlorinated organic chemicals are produced by bleaching in kraft mills. About 300 low-molecular-weight organochlorines have been identified in bleach plant waste, but they represent only 3% of the total weight of organochlorines.

Chlorinated phenolics and chlorinated resin and fatty acids account for much of the acute lethality of bleach plant effluent. Most of them are lethal to fish at about 1 mg/L or a few mg/L.

These low-molecular-weight substances are moderately bioaccumulative in fish, but are readily excreted if the organism moves to clean water. The chemicals are only moderately persistent in the environment, with half-lives estimated as a matter of a week, or a few weeks. Most of them are only partially removed by secondary waste treatment. High-molecular-weight chlorinated lignins persist for decades but are not very toxic.

D.6.1 General background

About 300 low-molecular-weight chlorinated substances have been identified so far in bleach plant waste, but the identified ones represent only about 3% to 10% of measured AOX (McKague 1988). About 80% of AOX consists of high-weight molecules (MW > 1,000 units) which have little toxicity. Although there has been concern that high-molecular-weight organochlorines might decompose to toxic low-molecular-weight ones, Kovacs et al. (1991) consider that to be unlikely because when the lignins and other high-molecular-weight organochlorines in BPME decompose, they tend to lose their aromatic structure which reduces the potential toxicity of derivatives.

Because the low-molecular-weight chlorinated compounds are of greater toxicological significance, there has been appreciable research in recent years to characterize that fraction (Kovacs et al. 1991). A major effort was carried out by Suntio et al. (1988) to list the known chemical and toxicological characteristics of some 250 chemicals from pulp mills, particularly the organochlorines from bleaching operations.

The chlorinated phenolics have received particular attention in the literature, although Kovacs et al. (1991) say that no biological effect has been demonstrated from their accumulation in aquatic organisms. These are also discussed under the topic of TEQ (Appendix F). The four major categories of chlorinated phenolics of interest here are phenols, catechols (2-hydroxyphenols), guaiacols (2-methoxyphenols), and vanillins (2-methoxy-4-formyl-phenols) (Fig. D.1).

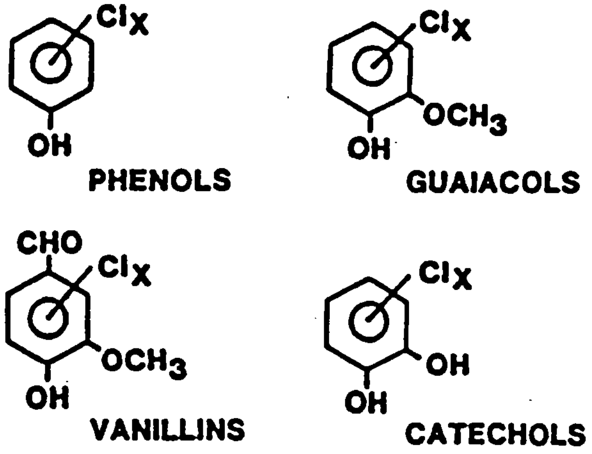

Fig. D.1. The four major classes of chlorinated phenolic compounds in effluent from bleached kraft mills using softwood. From Kovacs et al. (1991).

Chlorophenols show moderate resistance to decomposition, and removal of chlorophenols in secondary treatment ranges from zero to 40 % (Oikari and Holmbom 1986). Jones et al. (1991) reviewed other studies indicating similar removal rates of 12% to 50% for chlorinated phenols, catechols, and guaiacols, although one Canadian mill found removals of about 80% for chlorinated guaiacols and vanillins.

The half-life in natural waters would be about a week for most chlorinated phenols with 2 to 5 chlorine atoms, about a week for chlorinated catechols, and several weeks for chlorinated guaiacols, as predicted from laboratory experiments (Kuivasniemi et al. 1986).

Although most of the chlorinated phenolics cannot be considered as highly persistent, it is clear that many of them will pass through treatment plants and will be present in natural waterbodies. The more important toxicants listed in Table D.1 should certainly be appraised for water quality guidelines, and group or class TEQ systems would seem feasible, if desired (Appendix F).

Micro-organisms in the environment may transform chlorinated organics, and bacterial O-methylation may create chloroveratroles which are as lethal to larval fish as the parent chemicals, and can also cause deformities. Jones et al. (1991) have reviewed Swedish and other recent work on these substances. The transformed substances have been reported from water systems near Canadian pulp mills, and if future field studies found them in appreciable quantities, they might become candidates for water quality guidelines.

Chlorinated thiophenes represent another group of substances documented in bleach plant wastes. Thiophenes are similar to a benzene ring with a sulfur atom in the ring. They are lethal to crustaceans at a few mg/L, and some are mutagenic (Carlberg et al. 1988).

D.6.2 Toxicity of organochlorines in BPME

Ranges of toxicities and concentrations in effluents were provided by McLeay (1987) for the more important organochlorines, and the tabulation in Table D.2 includes practically all of the chlorinated organics which were listed earlier (Table D.1) as major toxicants. For several substances, the upper limits found in untreated effluent (third column of values) approach or exceed the lower limit given for lethal concentration (first column of values).

A similar list of organochlorines was made by Kovacs et al. (1984) for discharges from aerated lagoons at 9 Canadian bleached kraft mills. It showed average concentrations which tended towards the lower part of the ranges listed in Table D.2. Leach and Thakore (1975) evaluated the caustic extraction streams at 6 Canadian kraft mills, and found that the major chlorinated organics in those streams were at 4.3 times the lethal concentration, on average, assuming that their toxicities were additive.

The organochlorines account for a moderate amount of the acute lethality of untreated or treated BKME. They could be expected to contribute significantly to sublethal toxicity in some waters.

D.6.3 Bioaccumulation

The major organochlorines in BKME are moderately bioaccumulative in fish, but laboratory experiments indicate that they are excreted in a few days when fish are returned to clean water (reviewed in Jones et al. 1991).

Table D.2 Ranges of toxic concentrations (µg/L) and ranges found, for organochlorines in bleached kraft mill effluent (BKME). Adapted from McLeay (1987).

Organochlorine	96-h LC50 (µg/L)	Untreated BKME Conc.	Biotreated BKME Conc.
Dichlorocatechol	500-1000	12-90	1-120
3,4,5-Trichlorocatechol	1000-1500	120-270	2-280
Tetrachlorocatechol	400-1500	22-420	2-240
Dichloroguaiacols	2300	22-100	12-60
Trichloroguaiacols	700-1000	<10-340	<1-220
Tetrachloroguaiacol	200-1700	<10-620	<1-220
2,4-dichlorophenol	2800	9-15	2-51
2,4,6-trichlorophenol	450-2600	<1-51	<1-61
Chlorodehydroabietic a.	600-900	<10-750	<1-260
Dichlorodehydroabietic a.	600-1200	<10-410	<10-152
Dichlorostearic acid	2500	<40-552	<40-268

D.7 Recent chemical description of kraft effluents in Ontario

Summary. Recent measurements on effluents of nine Ontario kraft mills show that the average concentrations of resin and fatty acids, and low-molecular-weight chlorinated organics, are similar to those present in effluents a couple of decades ago. In the average of Ontario effluents, the sum of resin acids alone would appear to be lethal to fish, as would the sum of chlorinated resin acids and phenolics.

The monitoring of Ontario pulp mill effluents under the MISA program has produced some useful data. Nine kraft mills were surveyed to produce the data of Table D.3, and only four of them had secondary waste treatment at the time of the survey in the latter half of 1990.

Table D.3. Chemical measurements of resin and fatty acids, chlorinated phenols, resin acids, and certain other organic compounds, in effluents from nine kraft mills in Ontario during the second six months of monitoring under the MISA program (OMOE 1991). Substances marked with two asterisks were included as major toxic contributors in Table D.1, those with one asterisk were listed as lesser contributors.

Variable	No.[a]	F.d., %[a]	Min.	Max.	Average	Unit
Dehydroabietic acid**	668	84	.00	8.46	.44	mg/L
Abietic acid**	52	73	.00	3.50	.29	mg/L
Neoabietic acid	52	58	.00	1.90	.17	mg/L
Isopimaric acid**	51	69	.00	3.50	.23	mg/L
Pimaric acid**	51	59	.00	.83	.08	mg/L
Levopimaric acid	41	51	.00	.23	.02	mg/L
Oleic acid*	51	69	.00	.78	.11	mg/L
AOX	671	100	.04	135.	16.7	mg/L
Chlorodehydroabietic a.**	51	69	.00	.62	.08	mg/L
Dichlorodehydroabietic a.**	667	56	.00	.84	.04	mg/L
2-Chlorophenol	52	6	.00	.80	.03	µg/L
2,4-Dichlorophenol	52	46	.00	9.20	1.52	µg/L
2,6-Dichlorophenol	52	2	.00	.70	.01	µg/L
2,3,5-Trichlorophenol	52	21	.00	20.4	1.96	µg/L
2,4,5-Trichlorophenol	52	4	.00	7.30	.21	µg/L
2,4,6-Trichlorophenol	52	33	.00	15.2	2.13	µg/L
2,3,4,5-Tetrachlorophenol	52	2	.00	4.10	.08	µg/L
2,3,4,6-Tetrachlorophenol	52	2	.00	1.20	.02	µg/L
Pentachlorophenol	50	2	.00	1.20	.02	µg/L
Chloroform	51	98	.00	1880.	360.	µg/L
Phenol	52	42	.00	143.	17.3	µg/L
Toluene	49	43	.00	31.	2.60	µg/L

[a] No. = number of measurements. F.d., % = frequency of detection as a percentage.

A couple of instructive (although approximate) generalizations may be made from tabulations and comparisons of the recent data from Ontario: (1) concentrations of these compounds seem to be similar to historic data; and (2) concentrations for undiluted effluents still appear to be up into the lethal range.

The Ontario data in Table D.3 show zero or near-zero values for all of the chemicals, at some place or time during the survey. Values below detection limits were, however, assigned a zero value in the Ontario report and the detection limits were not stated. A few very high maximum values were obtained. Most average values were of appreciable magnitude, but some of the more highly chlorinated phenols, and the chlorinated resin acids, were at low concentrations in the hundredths of micrograms per litre.

The Ontario chemical data, which are very complete, may be compared with historic data, which are somewhat scanty judging by the review of McLeay (1987). This is done in Table D.4, based on U.S. samples for bleached kraft mills with and without biological treatment. In a couple of cases in the U.S. data, there are higher concentrations in the treated effluent than in the untreated waste, and that must be ascribed to sampling and perhaps analytical vagaries in the relatively low number of 15 samples for each type of effluent.

The Ontario averages are, for all substances, of the same order as the historic U.S. values. Many of the values fit very closely, considering that the Ontario averages represent a mixture of treated and untreated effluents. The comparison suggests that conclusions drawn from accumulated information in the literature should be relevant and realistic for present-day Canadian effluents.

Ontario kraft mill effluents would be expected to be toxic, judging by the recent chemical analyses, and that is generally found to be the case (Bonsor et al. 1988). The simple sum of the averages for 6 resin acids in the Ontario monitoring (Table D.3) is 1.25 mg/L, which is into the range of 1 to 2 mg/L for lethality of these materials for fish. The sum of the 9 chlorinated phenols is 6 mg/L, above the range of 1 to 2 mg/L for LC50s known for two such substances, as shown in Table D.2. Three of the chlorinated phenol averages for Ontario are, separately, into the range of 1 to 2 mg/L for expected toxicity.

Development of water quality guidelines for those chemicals in Table D.4 which lack them, would provide a useful base for evaluation tools in Canadian aquatic habitats. It would be especially appropriate to develop a system for evaluating the joint action of the similar chemicals.

Table D.4. Average concentrations of some resin and fatty acids and organochlorines in bleached kraft mill effluent for nine Ontario kraft mills monitored in the latter half of 1990 (OMOE 1991), compared with historic concentrations in 15 samples from U.S. mills* (Dellinger 1980). All values in $\mu g/L$.

Chemical	Historic U.S. values		Average values for 9 Ontario kraft mills
	Untreated	Biotreated	
Dehydroabietic acid	610	250	440
Abietic acid	700	380	290
Isopimaric acid	110	180	230
Pimaric acid	130	180	80
Oleic acid	790	71	110
Chlorodehydroabietic acid	67	23	80
Dichlorodehydroabietic acid	25	16	40
2,4-Dichlorophenol	3	2	1.5
2,4,6-Trichlorophenol	9	3	2.1
Chloroform	1500	8	1400

* The historic values are the weighted means for 6 samples from market bleached kraft mills and 9 samples from board, coarse and tissue bleached kraft mills.

D.8 Guidelines for classes of chemicals and individual chemicals

Summary. Canadian guidelines exist for classes of chlorinated phenols. It is desirable to use existing data to also develop general guidelines by class, for resin and fatty acids, the other major toxicological components in BPME. For many individual chemicals in BPME, it may not be feasible to establish guidelines without further toxicity testing.

Appendix C listed some Canadian water quality guidelines that apply to chemical components of bleached pulp mill effluent. Most notably, guidelines exist for chlorinated phenols, allotted to various categories according to degree of chlorination. Those substances constitute an appreciable portion of the list of toxic components of BPME (Table D.1).

Single-chemical guidelines that exist should certainly be used. If any guideline is exceeded, that clearly suggests potentially harmful conditions in the receiving water. However, even if no single guideline was exceeded, it would not necessarily follow that the receiving water was safe with regard to the total effluent that it contained, because of potential joint action of toxicants.

D.8.1 Developing new guidelines by class of compound

Recent compilations indicate that there may not be enough data to establish new water quality guidelines for individual chemicals, even for those that are toxicologically most important in BPME. Therefore, retaining general categories as the base of guidelines, as in the chlorinated phenols, appears to be a reasonably valid approach (section F.2). It might be desirable to review the factors assigned to the various chlorination classes, in view of some differences from a recent exercise of that kind done by Kovacs et al. (1991).

Considering that the chlorinated phenols already have guidelines, the obvious remaining major group of toxicants is the resin and fatty acids (Table D.1). It is recommended that Canadian guidelines should be developed for these substances, perhaps using an approach similar to that used by the Ontario Ministry of the Environment (Taylor et al. 1988).

D.8.2 Obtaining new technical information on individual chemicals

To remedy any lack of toxicity data among the most important chemicals in BPME, a straightforward approach would be to encourage or sponsor tests "to fill in the blanks". Encouragement of a testing program would appear to fit the mandate of Environment Canada and the Green Plan. The chief technical impediment to a testing program would be that not all the toxic substances in BPME are known. The merits of such a program should be compared to other potential uses of funding for environmental toxicology.

Toxicological data-gathering for a substance, with several kinds of organisms and a formal analysis and write-up, could be expected to take several months. Current costs for a sublethal whole-organism test would be close to $1,000, but several-fold higher for testing a particular chemical with verification of concentrations and all other aspects at a research level of quality. Allowing a modest doubling of costs for upgrading, meeting the minimum requirements of toxicity data for a freshwater guideline (CCME 1991) might cost $11,000. For 30 chemicals, costs might be $330,000, but allowing for difficulties of obtaining and working with some of the chemicals, half a million dollars might be closer to the cost.

APPENDIX E
PERSISTENT BIOACCUMULATIVE SUBSTANCES

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E.1 The rationale for guidelines based on body burdens

Summary. *Toxic lipophilic substances may be poorly assessed by water sampling, but well judged by tissue residues in resident organisms. This is commonly done using limits for use as human food, as for chlorinated dioxins and furans in B.C. coastal shellfish. The same principle can provide guidelines for aquatic organisms. A few body burden guidelines were listed in the U.S.A. two decades ago. A Canadian method for the derivation of tissue residue guidelines is currently under development.*

Some substances do not travel as molecules dissolved in water, but are sorbed onto particles, contained in particles, found in sediments, or accumulate in organisms. Indeed, some lipophilic substances may be difficult to find in water, or difficult to measure without appreciable error. Sometimes the apparent concentrations in water may fluctuate widely, depending on a number of extraneous circumstances such as degree of suspension of particles in relation to season or river flow.

For some very toxic, hydrophobic substances that accumulate in fish and other aquatic food organisms, it is an established principle to protect human consumers by imposing limits for tissue concentrations in the food organisms. In Canada the principle was put into use recently for marine areas polluted by chlorinated dioxins and furans. The pollutional status of such areas is judged, in fact, by those measurements in organisms and not by water samples. On the basis of residues in aquatic organisms, areas in the Strait of Georgia were closed to commercial fishing, and pressure was placed on pulp mills to reduce inputs of these contaminants.

Environment Canada is currently developing (a) a method for deriving tissue residue guidelines for the protection of wildlife which consume aquatic organisms, and (b) a method for the derivation of sediment quality guidelines, to address sediment-associated contaminants.

Tissue residue guidelines would differ from water quality guidelines in being suitable for different substances and for different purposes. The scientifically-derived tissue residue guidelines could be used for developing tissue residue objectives for specific localities. These residue objectives could serve as convenient benchmarks for interpretation in Environmental Effects Monitoring (tactic no. 3 of a control strategy, Appendix B). Unlike water quality objectives, it is unlikely that tissue objectives could be used in a predictive way to back-calculate from the environment to controls of discharge, because of the complexity of modelling such bioaccumulative substances at the present time.

E.2 Precedents for a tissue residue approach

It is common to limit human consumption of fish and shellfish, according to residues of strongly toxic substances, and the highly relevant example is the one mentioned above, of dioxin/furan closures on the coast of British Columbia.

As early as twenty years ago, it was conventional practice, for certain substances, to establish guidelines for the protection of aquatic organisms on the basis of body burdens, in addition to concentrations in the water. In the U.S.A., the freshwater aquatic life section of *Water quality criteria 1972* (NAS/NAE 1974) contained several such guidelines for very toxic bioaccumulative substances.

- Mercury had a tissue residue guideline as well as maximum and average limits for water.
- PCBs had residue and water guidelines.
- Certain pesticides had a tissue residue guideline, in addition to water guidelines, "to protect predators". This was done for Σ DDT, aldrin, dieldrin, endrin, heptachlor, chlordane, lindane, benzene hexachloride, toxaphene, and endosulfan. The limit was 0.1 mg/kg either singly or in combination.

E.3 Relevance for substances in pulp mill effluent

Summary. The assessment under the CEPA Priority Substances List did not point to any strongly toxic persistent bioaccumulative substances in bleached pulp mill effluent (aside from the dioxins/furans). Because of that, and inadequate information relating body burden to toxic effects, it does not appear feasible at the present time, to develop guidelines based on tissue residues for substances in BPME.

Environment Canada completed an assessment of toxic substances in BPME, including bioaccumulation (Jones et al. 1991). Some generalizations may be made from that assessment.

- There are relatively few substances in BPME that are strong, persistent bioaccumulative substances. The stronger accumulators are some of the more highly chlorinated phenolics, evidently some trichlorophenols, tetrachlorophenols, and tetrachloroveratrole, a transformation product of chloroguaiacol. Trichloro- and tetrachloroguaiacol and trichlorocatechol have accumulated in marine invertebrates.
- Many organochlorines are eliminated by fish in relatively short times of days or weeks if the fish move to clean water. However, continued exposure in a given location is the norm for aquatic organisms, leading to continuing body burdens.

E.4 Absence of toxicity information for developing guidelines

What is missing from the picture of body burdens of BPME constituents, is any cohesive review or statement about the association of concentrations in organisms with harmful effects on the organisms or their predators. Regulatory limits for human food intake are often used as cut-off points for levels in fish and invertebrates, since levels that are thought to be harmful to humans would also be expected to be harmful to predators. They might be particularly harmful to piscivorous birds which seem vulnerable to accumulating toxicants.

The literature on effects of BPME components apparently does not provide a diagnosis of degrees of effect that are caused by bioaccumulation of the chlorinated phenolics. Lacking such identification of toxicity, guidelines cannot be developed at present, on a basis of body burdens of organochlorines or other toxicants in BPME.

The exception to this lack of data and guidelines on harmful body burdens, is the limit for compounds belonging to the families of chlorinated dioxins and furans. In several Canadian fisheries, human consumption limits have been applied for those substances, however those substances are not to be included in the present report.

E.5 Expert advice

Support for concept
Dr. John Ward and Nadene Henderson (B.C. Ministry of Environment) pointed out that they intend to develop an approach which starts with a human health guideline for a particular substance in fish, and models back through its water carriage, to an assessment of loading level from an effluent. They are tentatively aiming at guidelines for 10 or 20 specific substances, and perhaps for fractions of AOX. This is further discussed under modelling (Appendix K).

Negative comments

Lord Crickhowell of the U.K. National Rivers Authority did not support guidelines on body burdens. He thought in terms of regulation and control, and for dangerous bioaccumulative substances, simply stated that they should be controlled by a best-technology approach. The Commission of the European Communities operates in largely the same fashion, by limiting discharge of dangerous pollutants (CEC 1990). Reduction at point of discharge is indeed the proper mechanism for control (i.e. tactic no. 1), but in addition, tissue residue guidelines provide the scientific background for judging whether control seems satisfactory, since body burden objectives derived from the guidelines can serve for monitoring (tactic no. 3).

EOX as an overall indicator

Extractable organic halogen (EOX) might serve as an index of total organochlorines in an organism. This might become useful if there were progress in defining harmful effects associated with the residues. The measurement suffers from the same problems as AOX, since a given value could be caused by particularly harmful substances, or by non-harmful ones.

A major problem with this approach is that organisms customarily convert toxic chlorinated phenols into less harmful substances. Dr. Bruce McKague (Pulp and Paper Centre, University of Toronto) pointed out that one can only explain a small fraction of EOX in terms of known substances from the effluent. For example, fish convert dichlorostearic acid (a long-chain fatty acid) and store it as a glyceride [?]. Chemical analysis of EOX is therefore not very revealing; instead one should know in detail what substances were present.

Dialysis tubes as standard sampling devices

I.H. Rogers of Fisheries and Oceans (Sidney B.C.) felt that anyone concerned with developing guidelines should be aware of a potential exploratory/monitoring tool that was currently the subject of research. This was the use of "surrogate fish" in the form of dialysis tubes containing lipid. The ease of harvesting such devices would have obvious advantages for orienting control efforts. A further obvious benefit would be that the investigator would know the "exposure history", which is seldom the case for fish which may move in and out of an area. Guideline development would, of course, be concerned with living organisms.

APPENDIX F
TOXICITY EQUIVALENTS

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Summary. The presumed joint effect of several toxicants belonging to a single category may be expressed as one number by using factors based on toxicity, relative to the most toxic substance in the category. The "TEQ" system is generally accepted for chlorinated dioxins and furans. A similar system is available for the chlorinated phenolics in pulp mill effluent.

If a large number of similar toxic chemicals occur together, one way to simplify the evaluation of their effects is to use the method of *Toxic Equivalents (TEQ)*. This is a possibility if the substances belong to the same general chemical class, and in particular if they have the same mode of action or similar modes.

The method adopts one chemical as the standard for the group. The most toxic one is the logical choice as a standard. Each chemical in the group is given a rating or *toxicity factor* for its degree of toxicity. Then the amount (or concentration) of a given substance is expressed in terms of the *equivalent* amount of the standard substance, i.e. for an equal toxic effect. The equivalent amounts (TEQs) for all substances are added together to give a single value that can be worked with more easily.

The approach is a variation on approaches used in assessing toxicity of mixtures (see Appendix G). In particular it is a variation of the Toxic Units method, since it assumes simple addition of the toxic effects of various chemicals within a group. The TEQ method has received general acceptance by scientific and regulatory communities for appraising mixtures of chlorinated dioxins and furans. Although the method is similar to the toxic units method, it is convenient to discuss in this separate section, some attempts at developing systems for TEQ. As an example, the system used with dioxins is outlined below.

F.1 A pattern to follow. TEQ for dioxins and furans

Summary. The TEQ system for chlorinated dioxins and furans is accepted internationally. Eleven factors are used to express the amounts of individual chemicals as the equivalent amount of the most toxic dioxin.

The "dioxin" of popular usage can be translated as 210 chemicals belonging to two families. There are 75 different polychlorinated dibenzo-p-dioxins *(PCDDs)* and 135 different polychlorinated dibenzofurans *(PCDFs)*. All of them have a similar basic structure with two benzene rings and a specific type of linkage incorporating oxygen.

The various members of these families differ in the number of attached chlorine atoms, and in the positions of those chlorines. Toxicity tends to increase with increasing number up chlorine atoms up to four [the tetrachlorodibenzo-p-dioxins *(TCDDs)* and tetrachlorodibenzofurans *(TCDFs)*]. Toxicity decreases as more chlorines are added, through the series with 5 chlorines (penta), 6 (hexa), and the relatively non-toxic 7 (hepta) and 8 (octa) forms. The chlorinated furans are less toxic compared to chlorinated dioxins. The octachlorinated dioxins and furans are often more abundant than other forms, but have practically no toxicity.

The two important members for toxicity are 2,3,7,8-tetrachlorodibenzo-p-dioxin *(2378-TCDD)* and 2,3,7,8-tetrachlorodibenzofuran *(2378-TCDF)*. The most toxic is 2378-TCDD, and other isomers are expressed as approximate TEQs of that substance, by applying appropriate factors.

The TEQ system developed during the 1980s as information accumulated on the relative toxicity of the various dioxins and furans. Various tables of values for toxicity factors were promulgated, for example by U.S. EPA and in a very detailed report from Ontario (reviewed in Bonsor et al. 1988). However, it is a measure of the acceptance of the TEQ method that there is now general recognition of a standard international system published by NATO (1988). Some Canadian provinces seem to be adopting the NATO system, for example it is used in Alberta.

The international factors for dioxins and furans (Table F.1) are not of direct use in this report, but they are given as an example of an accepted system and as a possible pattern for TEQ systems for other families of chemicals. To use the system, the weight (or concentration) of a compound measured in a mixture is multiplied by its toxicity factor, listed beside it. The result is the approximate equivalent of 2378-TCDD. After calculating equivalents for each of the components of a mixture, they are added up to yield the total Toxicity EQuivalent or TEQ of 2378-TCDD. The total TEQ is the **prediction** of the overall toxicity of the mixture. It is important to remember that the NATO system is intended to have a base of mammalian toxicity; recent research shows that for aquatic organisms, relative importance of the different congeners changes, and different toxicity factors are appropriate.

All the substances with an assigned toxicity factor for TEQ have chlorines on the 2, 3, 7 and 8 positions of the molecule. NATO (1988) uses the term *International Toxicity Equivalency Factors (I-TEFs)*. In the present report, the less formal term *toxicity factors* is used instead, since with pulp mill effluents, the abbreviation TEF signifies "Toxicity Emission Factor".

Table F.1. Toxicity factors for chlorinated dibenzo-p-dioxins (CDDs) and chlorinated dibenzofurans (CDFs), as used internationally (NATO 1988).

MCDDs	(Monochlorinated)	no toxic allowance
DCDDs	(Dichlorinated)	no toxic allowance
TrCDDs	(Trichlorinated)	no toxic allowance
2378-TCDD	(the standard)	1.0
other TCDDs	(other tetra CDDs	no toxic allowance
12378-PeCDD	(penta CDDs)	0.5
other PeCDDs		no toxic allowance
2378-substituted HxCDDs	(hexachlorinated)	0.1
other HxCDDs		no toxic allowance
123478-HpCDD	(heptachlorinated)	0.01
other HpCDDs		no toxic allowance
Octa-CDD	(octachlorinated)	0.001
2378-TCDF	(tetrachlorinated)	0.1
other TCDFs		no toxic allowance
12378-PeCDF	(pentachlorinated)	0.01
23478-PeCDF		0.5
2378-substituted HxCDFs	(hexachlorinated)	0.1
2378-substituted HpCDFs	(heptachlorinated)	0.01
Octa-CDF	(octachlorinated)	0.001

F.2 TEQ systems developed for pulp mill effluent

Summary. A TEQ system has recently been developed for the chlorinated phenolics of pulp mill effluent. It is based on sublethal toxicity to aquatic animals. Some of the toxicity factors fit well with existing Canadian water quality guidelines.

Toxicity factors have been assigned to chlorinated phenolics in BPME by Kovacs et al. (1991). Four major classes of phenolics were designated as the phenols, catechols, guaiacols and vanillins (Fig. D.1). There was a general tendency for greater toxicity of substances which are more highly chlorinated (3 to 5 chlorine atoms per molecule).

Kovacs and colleagues based their analysis on the literature for sublethal effects only, and imposed some other astute limitations on the information used. The number of tests available was small (8 studies) but the three major relationships that emerged showed convincing agreement. An assumption was necessary, that substances from the four classes that had the same degree of chlorination would have the same degree of toxicity, but the authors present separate information supporting that assumption. The authors say that the TEQ relationship should be regarded as provisional, but it appears likely to be supported by future results. Taking pentachlorophenol as the base of the system (most toxic), the other degrees of chlorination show toxicity factors that range downwards two orders of magnitude (Table 1).

There are some similarities of the values estimated by Kovacs et al. (1991) with the earlier assignment of relative toxicities for Canadian water quality guidelines. Table 1 also shows the guidelines published by CCREM (1987). The values may be converted to toxicity factors as indicated in Table 1. The pair of factors for tetrachlorophenols are essentially identical, while the other pairs differ by factors of 2, 4, and 8. An examination of the reasons for the larger differences might be in order, but the general correspondence is encouraging.

The TEQ approach was carried further by Holloran et al. (in press). They used three systems of TEQ for three categories of substances. For the first category, they assigned toxicity factors to all groups of chlorinated phenolics, using preliminary values of Kovacs et al. (1991). Into this first category they added certain unchlorinated substances (phenol, cresol, guaiacol, catechol and veratrol) by assigning each substance a factor of 0.001. For the second category they assigned factors ranging generally from 0.2 to 0.75 to 9 resin acids, relative to a value of 1.0 for the most toxic one, isopimaric acid. For the third category they assigned similar factors to 10 fatty acids, relative to a value of 1.0 for the most toxic, chlorostearic acid. The database for the resin and fatty acids was not detailed.

Holloran et al. (in press) achieved predictions of effects that seem reasonable. They summed within the three classes (phenolics, resin acids, fatty acids) but did not add between classes. There was practical benefit in being able to make general predictions of toxicities, as a way of summarizing the rather long lists of measured chemicals in various pilot-plant effluents. The exercise was useful in planning a mill in Alberta that would meet environmental requirements.

Sublethal tests (with *Ceriodaphnia*) were done for five pilot-plant effluents by Holloran et al. (in press). These actual measurements of toxicity may be compared with the toxicity predicted by the TEQ systems (i.e. derived from chemical measurements in the effluents). For the five effluents, I calculated single TEQ values by summing the TEQs for phenolics, resin acids, and fatty acids with appropriate weighting for the actual toxicity of the reference substance in each category. On average, the effluents were 2.1 times as toxic as predicted (individual ratios 1.1, 1.7, 2.0, 2.6, and 3.0 times as toxic as predicted)[8]. That is not bad correlation for aquatic toxicology (Sprague 1985) considering the exploratory nature of the work. The TEQ system and the principle of summing toxicity between categories of substances, seems to hold some promise for future development, judging by this work of Holloran et al. (in press).

F.2.1 Expert advice

Summary. Most people interviewed did not discuss the TEQ approach; most of those that did suggested caution because of a lack of knowledge of what substances were involved and their toxicity. There was general approval, however, of the toxicity factors for chlorinated phenolics.

Of the 20 people interviewed specifically for purposes of this report, almost half did not suggest the topic of TEQ, and about one-quarter of the respondents suggested caution in applying this concept to pulp mill effluent. The discussions remained at the level of predicting the toxicity of effluents, and application to developing guidelines would be a further step.

Some positive responses were obtained, however. One chemist liked the TEQ approach since one can use a single-run chemical procedure to reveal a whole class such as the chlorinated phenols (Dr. Bruce McKague, Pulp and Paper Centre, University of Toronto). The system developed by Kovacs et al. (1991) for chlorinated phenolics met with general approval.

Some people felt that our chemical knowledge was not yet satisfactory to apply the TEQ technique, even for predicting toxicity of effluents. Some standard comments may be paraphrased as follows.

- We do not know what substances are causing the problems, so we cannot apply this system.
- Effluents are not characterized to the extent necessary to use a TEQ approach. New processes produce a great variety of substances, and even the chlorinated phenols may not be there in the future, to assess by TEQ.
- There is a shocking lack of toxicity information on specific substances.
- A classification based on water-borne concentrations could have problems. It would be better to have a system based on dose in fish.

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[8] Part of the calculation involved an appropriate chronic/acute ratio of 0.1 (Bonsor et al. 1988) instead of 0.2 which was used by Holloran et al. (in press).

F.3 AOX as an unsuitable method of assessing toxic effects

Summary. Adsorbable organic halogen measured in bleached pulp mill effluents is not well correlated with toxic effects of the effluents, particularly for today's lower levels of chlorination.

Scandinavian research in the 1980s gave general and specific indication that toxicity increased in pulp mill effluents, with the degree of chlorination used in bleaching (reviewed in Sprague and Colodey 1989, Jones et al. 1991). There was a need for some convenient chemical measurement which would describe the chlorinated substances in effluent.

Adsorbable Organic Halogen (AOX) is a simple system to integrate diverse chlorine-containing organic substances, from small to large molecular weight (Fig. F.1). The single measurement does not, however, represent a realistic evaluation of toxicity of a class of compounds. It is covered here for completeness, because the AOX measurement is well known.

AOX is simply a measurement of the amount of halogen contained in organic molecules in the sample; for pulp mill wastes chlorine would be essentially the only halogen present so AOX is a measure of chlorine attached to organic substances. It is measured by the amount of chlorine that adsorbs onto a carbon column during the analytical procedure.

It might be assumed that if a pulp mill chlorinated heavily, there would be high amounts of toxic organochlorines in its effluent, it would be very toxic, and this would be reflected in high values for AOX. The assumption probably holds for very high levels of AOX in BPME. At the lower levels of chlorination seen today, any such relationship is so rough as to be of little value.

An AOX measurement for BPME would probably include hundreds of diverse chemicals, many of them with low toxicity, for example, high-molecular-weight organochlorines that have little or no toxicity. Only about 1% of the AOX represents lipophilic substances of toxicological concern (see Fig. F.1). AOX should now be considered an indication of the degree of chlorination at a mill, and/or the degree of waste treatment, and not an indicator of toxic effects.

F.3.1 Evidence on unsuitability of AOX

The **toxicity** associated with a reduction in AOX decreases much more precipitously than does the AOX itself, when bleaching processes are changed to use less chlorine. Fleming et al. (1990) estimated that as AOX decreased from 5.7 kg/t to 3 kg/t (less than a 2-fold drop), the total toxic effect of organochlorines in the effluent decreased from 100 to about 18 TEQ (more than a 5-fold drop). A more recent summary of findings shows a decrease of about 15-fold in total toxicity of chlorinated phenolics for a 3-fold drop in AOX (Fig. F.2). Similar conclusions were reached by Holloran et al. (in press) who studied effluents from pilot-scale bleach plants. Comparing conventional bleaching to no-chlorine bleaching, they concluded that an 84% reduction in AOX was associated with a 99% reduction of toxicity of chlorophenolics.

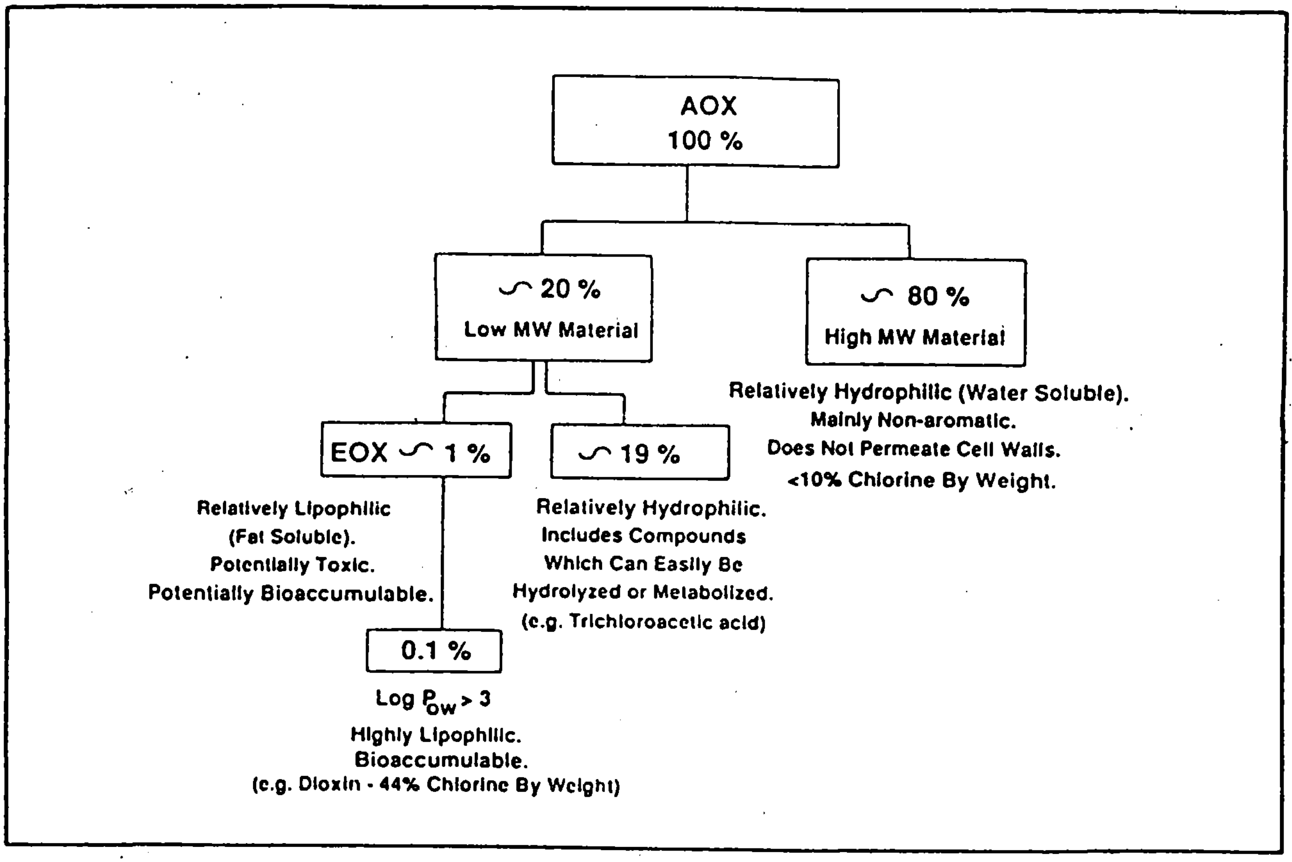

Fig. F.1. Sub-categories of AOX in effluent resulting from production of conventional bleached kraft pulp. From Berry et al. (1991).

Chemical measurements substantiate the strong decreases of toxicity. Concentrations of chlorinated phenolics in effluent decrease much more precipitously than does AOX. Smith and Sprague (1992) show generalized data that AOX may decrease 2- to 3-fold for mills that change their process from bleaching mostly with chlorine to high substitution with chlorine dioxide. However, a comparison of the chlorinated phenolics in the effluents from those two types of bleaching, showed much larger decreases of 14-fold in the phenolics of the wastewater before it was treated, and 60-fold in treated effluent.

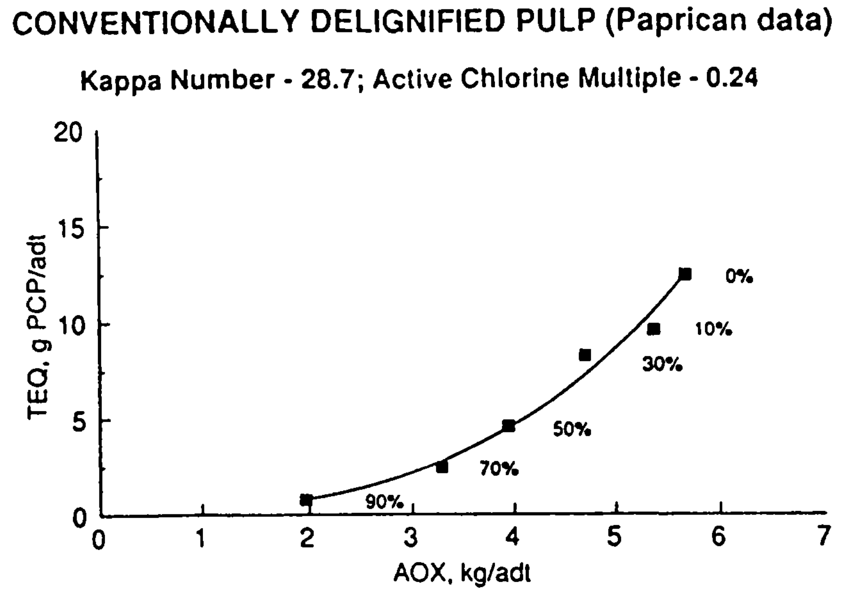

Fig. F.2. Decline of summed toxicity of chlorinated phenolics (TEQ) with decreasing AOX in effluent. Percentages in the body of the graph indicate degree of substitution of chlorine dioxide for chlorine. These data obtained by PAPRICAN correspond to a general relationship with dozens of data-points. From Berry et al. (1991).

The organochlorines of most concern, the dioxins, appear to show a particularly dramatic decrease as AOX declines. Fleming et al. (1990) also assessed the dioxins, and concluded that 2,3,7,8-TCDD which would be about 24 ng/L in bleach plant effluent for conditions of no substitution of chlorine dioxide and a high "chlorine multiple" (strong bleaching), would drop off quickly with 20 and 40% substitution, and drop to about 0 ng/L for 60% substitution and a moderate chlorine multiple. This fits with the experience of the E.B. Eddy mill in Ontario, which found that "50% chlorine dioxide substitution prevents dioxin formation" in their mill (C.R. Cook, Environmental Manager, pers. comm., January 1990).

Direct experimentation has recently become available from research at PAPRICAN (O'Connor et al. 1992), and it shows that neither lethal nor sublethal effects showed good correlation with AOX or EOX (extractable organic halogen). The comparisons were based on laboratory tests with the early life stages of fathead minnows, and also on reproductive success of daphnids. While a weak relationship might be read into such results as those in Fig. F.3, the correlation coefficients were poor. In particular, the sublethal effects caused by effluents from mills that bleached were similar to effects from some unbleached effluents. What Fig. F.3 really indicates is that secondary treatment is associated with lower toxicity, as marked off by the horizontal lines. Only one mill with secondary treatment falls in with the higher toxicity of the mills with primary treatment, and that mill probably had a treatment facility which was not functioning very well.

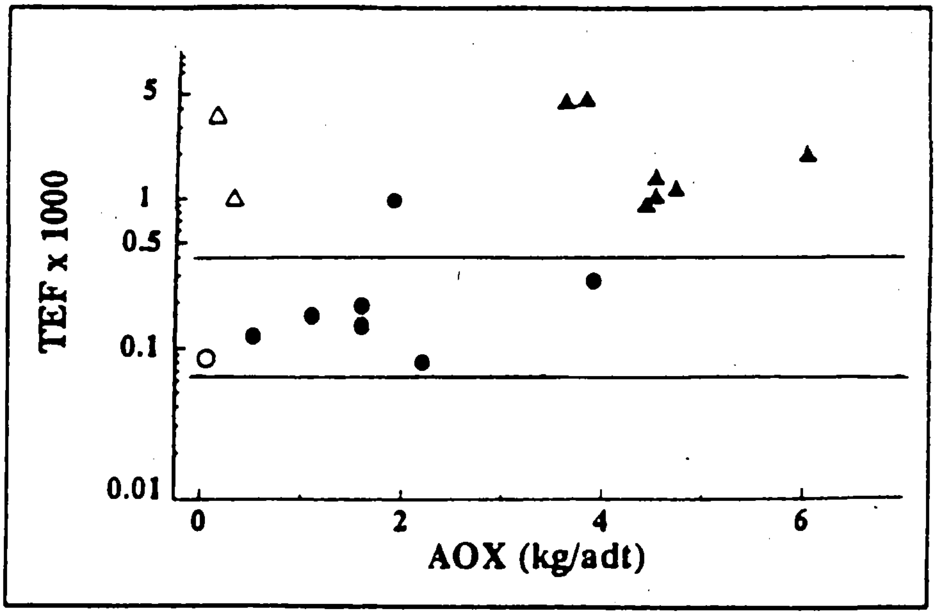

Fig. F.3 Poor correlation of sublethal toxicity with AOX in effluent. Toxicity was assessed by growth of larval fathead minnows. Toxicity is measured as Toxicity Emission Factor (TEF) and increases vertically. Eight mills are represented: triangles for primary treatment of effluent; circles for secondary treatment; black points for bleaching; open points without bleaching. The horizontal dashed lines have been added to show the grouping of all mills that had secondary treatment, with one exception. From O'Connor et al. (1992).

A similar result was obtained in work with U.S. effluents from kraft mills (NCASI 1989b). No correlation was obtained between AOX and 39 lethal and sublethal toxicity tests with fathead minnows and *Ceriodaphnia*. The slope of the attempted relationships was never different from zero.

Concerning AOX as an environmentally meaningful measurement, I questioned Scandinavian biologists in June of 1990 at the 3rd International Forest Industries Wastewater Conference in Finland. In general they cautioned against trying to correlate level of AOX *per se* directly with biological effects. People such as Anders Södergren, who headed the study Environment/Cellulose, said that once a mill reduced AOX levels to 1.5 to 2 kg/t, there was a disappearance of any association of effect with AOX. Toxic contribution of organochlorines, if any, could not be distinguished from the general effects of "traditional" pollutants such as BOD, resin and fatty acids, etc.

Another long-time Scandinavian worker on pulp mill effects has stated in a publication: "No significant correlation between TOCl [similar to AOX] ... and environmental impact seems to occur at TOCl levels below 1 - 1.5 kg per tonne of pulp" (Lehtinen et al. 1990). The final paragraph of the discussion in this paper states: "From the study it can be concluded that at discharge levels of TOCl below 1.0 - 1.5 kg per tonne of pulp [about 1.3 - 2.0 AOX] the TOCl-parameter does not seem to be of particular relevance in predicting environmental impact. This conclusion becomes even more evident when comparing results of other workers having tested effluents from production of unbleached pulp (softwood and harwood [sic]) on fish (Oikari et al. 1984, Bengtsson et al. 1988). From these studies it can be seen that effluents from unbleached production induce effects at least at an equally high degree as effluents from bleached pulp production."

New evidence from Ontario fits the recent trend to downgrade the importance of organochlorines as the component of BPME that caused sublethal "Swedish symptoms" in fish (induction of MFO enzyme systems and decrease in reproductive steroids). Surveys at 10 Ontario mills showed induction of MFO in fish below all mills, whether those mills bleached or not. (Dr. K.R. Munkittrick, Dept. of Fisheries and Oceans, Burlington, Ontario, pers. comm.).

F.3.2 "Fractionated AOX"

One idea is to filter the sample and measure only the smaller, more toxic organochlorine molecules in the filtrate. Fractionated AOX is intended to have molecular weights ≤ 400; that would go through a biological membrane, but would contain the chlorinated guaiacols and dioxins. This was ventured as a potential avenue of investigation, and one scientist expressed guarded optimism that we might be able to find a fraction of AOX which predicted toxic effects (Dr. W.L. Lockhart, Fisheries and Oceans, Winnipeg, pers. comm.). So far there is only a small amount of experimental evidence on the usefulness of the concept.

One set of well-controlled toxicity tests is available on this topic (O'Connor et al. 1992). Both lethal and sublethal tests were done, and they failed to discern any better correlation of toxicity with fractionated AOX (MW < 1000) than with total AOX. Neither measure showed appreciable correlation with toxicity.

APPENDIX G
TOXICITY OF MIXTURES

> *Since ... exposure to multiple chemicals appears to be the norm rather than an occasional event, guidelines must be developed in order to deal with this problem.*
>
> Denise Foulon, Health and Welfare Canada

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The above quotation from Foulon (no date) refers to human exposures, but is also relevant for aquatic pollutants. In real effluents and receiving waters there would almost always be more than one toxicant, usually several toxicants and often many. Nevertheless, research is scarce on the topic of toxicity of pollutant mixtures to aquatic organisms. Most of the tens of thousands of published studies in aquatic toxicology deal with only a single toxicant [9].

Several models have been proposed for evaluating mixed toxicants, but they continue to be relatively under-utilized or untested, as a consequence of the paucity of research [10]. That is unfortunate, considering the importance of the topic.

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[9] Apparently the scarcity of work on mixture toxicity is general, and not limited to aquatic toxicology. Foulon (no date) seemed puzzled that a literature search in the public health field did not provide a large number of references. She concluded that either the reports could not be retrieved by the key words (which seemed very logical ones), or that "there is very little literature concerning this topic".

[10] A review concerning zinc, lead and cadmium and marine shellfish (Sprague 1986) may be cited as an example. In all, 960 papers were reviewed for the project. There were lots of lethal tests of single metals, for example 47 determinations of the lethal concentrations of cadmium, an example of wasteful repetition. Yet among all the 960 papers, there were only 8 that considered toxicity of any one of the three metals in combination with any other metal.

Documents on water quality guidelines are customarily written in terms of single toxicants. Compendia of guidelines seldom give guidance on what to do when one must evaluate several simultaneous pollutants (U.S. EPA 1976), although tentative suggestions were once given (NAS/NAE 1974). Canadian guidelines (CCREM 1987) fit this pattern, with no advice on approaches to be taken with mixed pollutants; Appendix 4, which is devoted to guidance on factors that should be considered when using the Canadian guidelines to establish local or site-specific water quality objectives, does not cover toxicity of mixtures. The International Joint Commission proposed a water quality objective (IJC 1980) for mixtures of metals in the Great Lakes, based on simple additivity of individual metals (see toxic units concept in section G.2).

A trend toward dealing with the question may be developing in recent years. EIFAC (1987) proposed that the joint effects at higher toxicity levels should be assessed by assuming additive action for even diverse toxicants [11]. In the field of occupational hygiene, it is almost standard in protecting humans from airborne pollutants, to assume addition of effects of similar substances acting jointly (see section G.7).

G.1 Models and terminology

Summary. Some classical and modern models of joint action appear to require a knowledge or assumption of the mode(s) of action before the type of joint toxicity can be named and the joint effect predicted. An empirical system of nomenclature is recommended, which describes joint toxicity by one of the terms: supra-additive; additive; infra-additive; no addition; *or* antagonism.

The following descriptions are largely derived from Sprague (1970, 1990a) and from the explanations of Broderius (1991). Combined effects are often divided into four categories on the basis of their physiological action, and these are the ones that have been passed down from classical work on the subject by Hewlett and Plackett (1979) and others.

Similar.	The site of action within the organism is the same for both substances.
Dissimilar.	Sites are not the same.
Interactive.	One toxicant influences the biological action of the other, i.e. strengthens, weakens, or otherwise changes it. This may arise from changes in absorption of one toxicant, or distribution, biotransformation, or excretion. These relations are complex, relatively rare, and seldom dealt with in evaluating joint toxicity.
Noninteractive.	One does not influence the other. This is the common finding or assumption in almost all of the analyses of joint toxicity.

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[11] EIFAC did an extremely thorough review of laboratory and field studies of mixture toxicity using lethal effects as an end-point. Their analysis of sublethal effects was less searching, and their recommendations are very tentative for dealing with mixtures of substances at sublethal levels.

Thus the various models decrease in practice to two:
noninteractive similar action; and noninteractive dissimilar action.

In practice, the "noninteractive" is omitted, being common to both types. The two types of joint action commonly considered are variously named as follows.

Similar action, previously called *simple similar action*, and now sometimes called *concentration addition*. In this category, equivalent amounts of one toxicant can substitute for another.

Dissimilar action, a term which is seldom seen, previously called *independent action*, and now sometimes described by the apparently inaccurate term *response addition*. (The responses do various things, but do not add together.) Neither toxicant enhances nor interferes with the other.

Use of the above classification depends on knowing the mode of action of each toxicant, and that is often not the case. It would be desirable to know the physiological actions of all toxicants on aquatic organisms, since it would improve the chances of predicting patterns of joint toxicity. To some extent that can be done with QSARs, but biochemical/physiological knowledge will never be complete. The problem of assigning and allowing for modes of action becomes more complex as more substances are present in the mixture, and more modes are represented.

Accordingly, the classification scheme used in this report is based on the observed magnitude of "co-operative toxic action", without any particular classification by mode of action. The actions may be named according to the degree of whole-organism effect caused by a mixture in relation to effects caused by the individual components acting alone.

Terminology can be illustrated by the possibilities for joint effects of two substances as shown in Fig. 2. This figure and the associated explanations apply only to binary mixtures, but the principal can be applied to mixtures of many substances by using a mathematical equivalent.

Some comments follow, on the terms used in Fig. 2. Thinking only of toxicant A on the horizontal axis, 1.0 on that axis would represent the concentration that caused the (specified) effect. This might be the LC50, i.e. the concentration estimated to cause 50% mortality of organisms, or the EC50, for example the concentration estimated to cause reproductive failure in 50% of the test organisms. Alternatively, it might be the concentration estimated to cause 25% reduction in the growth rate of an alga. Similarly, 0.5 on the axis would be one-half of the concentration required to cause the effect. A value of 1.5 on the axis would be 1.5 times the concentration required to cause the effect. Exactly the same scheme is used on the vertical axis, i.e. 1.0 would be the concentration of toxicant B which caused the same endpoint.

Addition is represented on the diagonal of the diagram. If an experiment with two substances (A and B) acting simultaneously, yields a result which plots on the diagonal of Fig. 2, then the joint action is simple addition. An extreme example which helps to convey the concept, is to assume that toxicant A and toxicant B are actually the same substance. In that case, 0.5A + 0.5B would indeed be expected to produce the effect, i.e. 0.5 + 0.5 = 1.0. Similarly, if 0.1A with 0.9B produced exactly the effect being used as an endpoint, then A and B would be acting in a simple additive manner.

Supra-additive effects or more-than-additive effects would be represented by any results of an experiment that fell into the lower left triangle of the diagram, e.g. if 0.5A + 0.2B produced the effect (EC50 or whatever), they would be showing supra-additive joint action. This is sometimes called "potentiation", particularly in pharmacological circles, although it should be noted that potentiation could involve a chemical that did not itself produce the effect of interest, but increased the action of another chemical.

Infra-additive effects or less-than-additive effects would be represented by any results of a joint toxicity test which fell into the upper right triangle of the diagram, for example if it required 0.8A plus 0.7B to just produce an EC50. The substances would still be co-operating to some extent in causing an effect, but not in a fully additive way.

All three categories above represent "joint action", i.e. two toxicants work together in some degree, causing an effect that neither would cause by itself at the same concentration. "Synergism" also includes all three cases according to its dictionary meaning, but the term might be avoided since it is often taken to mean supra-addition.

No addition would be shown if the results of a test fell on the upper and right borders of the rectangle. That gives the appearance of each toxicant operating independently of the other, without influencing the action of the other. For example, there would be no addition if, in the presence of 0.3B, it still required 1.0A to cause the endpoint.

No addition could be considered a special case of infra-addition. In some publications, authors have considered this to be one case of "response addition" (the case in which tolerances of individual organisms to the two toxicants are perfectly correlated). As mentioned above, this seems to be difficult from the point of view of terminology since there is no apparent addition of effects along the borders of the rectangle.

Antagonism would be taking place if the presence of both toxicant A and toxicant B actually made the observed effect less than if one or other of the toxicants were present by itself, at the same concentration as in the mixture. This term is often misused, as if it meant infra-additive. The term should be used only in its proper sense, that one substance actually "antagonizes" or counteracts the other, i.e. makes its effect weaker.

G.2 Toxic units as a simple model of joint toxicity

Summary. The toxic units system may be used to assess the kind of joint action exerted by a mixture, or to predict what the effects of a mixture would be. The concentration of each chemical is expressed as a proportion of its effective concentration (or its water quality guideline), and the proportions are added together. The assumption is that a sum ≥1.0 would cause an effect.

One method of evaluation that was recommended in the past for practical application, was based on an assumption of additive action by different toxicants, often called the "toxic unit" method. This was the method given in a U.S. book of water quality guidelines (NAS/NAE 1974) and in a booklet of guidelines from the Ontario government (OMOE 1978). The system continues in standard use for protection of humans, in industrial exposure to substances of similar action (ACGIH 1988). In aquatic regulations, however, the method has disappeared from Ontario's documents (OMOE 1984). The U.S. "technical support document" (U.S. EPA 1991) recommends that acute toxicity from a number of substances should be considered additive, but that sublethal (called "chronic") toxicity should not be considered additive, because of lack of information that this is so.

In the toxic units system, the concentration of each toxicant is expressed not in chemical units, but as a fraction of its threshold of sublethal effect, or of its water quality objective/guideline, i.e. in *sublethal toxic units (SLTU)*. This would use the following formula.

$$SLTU = \frac{C_A}{TEC_A}$$

where SLTU	=	Sublethal Toxic Units
C_A	=	Concentration of Toxicant A that is actually present in the water
TEC_A	=	Threshold Effect Concentration of Toxicant A (or No-Observed-Effect Concentration, or Water Quality Guideline for Toxicant A)

The concentration must be in the same units for top and bottom of the fraction (e.g. mg/L). The effect could be any designated real change. In developing guidelines the effects studied would typically be reproduction, growth, development or survival. The endpoint might be an EC50 or a statistically significant EC10, or the IC25, the concentration resulting in a 25% inhibition in reproductive rate of an alga. The system could be used on a variety of effects such as the concentration which caused half of the organisms to show an avoidance reaction.

The toxic units approach to evaluating a mixture of Toxicants A, B, C, D, etc. would simply be an extension of the formula above. The proportion is calculated for each toxicant in the mixture, and those values are added together.

$$SLTU = \frac{C_A}{TEC_A} + \frac{C_B}{TEC_B} + \frac{C_C}{TEC_C} + \frac{C_D}{TEC_D} \quad etc.$$

Obviously, if a single chemical reached 1.0 SLTU, it would be at a threshold of concentrations causing sublethal effect. If the designated effect (just) occurred when all the components added up to 1.0, then the mixture would be showing additive effects. If the effect occurred only when the sum of the equation was significantly greater than 1.0, say 1.5, the effects would appear infra-additive, i.e. still having a joint ("co-operative") action but not as strongly as complete addition of effects. Conversely, if the effect occurred when the sum of the equation was less than 1.0, the effects would be supra-additive, e.g. if 0.1A + 0.1B + 0.1C caused the designated effect.

The boundary of antagonism is less definite. Certainly if it were determined that the joint effect could only (just) be obtained when one or more of the individual fractions was significantly greater than 1.0, then that would be antagonism.

All of the above explanation is concerned with a more or less experimental mode, i.e. how much is required to (just) attain the designated effect. The procedure can be reversed to yield a predictive mode. For each component of a mixture, the observed concentration is expressed as a proportion of the effective concentration. The proportions are then added and the total is interpreted on the basis of an assumed pattern of joint action. The usual assumption is additive action, i.e. if the sum of the individual components equalled 1.0 or greater, the designated effect would be expected. The assumption that actions of different substances are exactly additive is certainly not true in all cases but is often approximately so (see section G.3). The method is likely to be over-protective since toxicants at sublethal levels tend to show additive to less-than-additive joint action.

The toxic units model described here is based on a single defined level of response such as the concentration affecting the median organism in a group (EC50), or the EC10, or IC25 for a 25% impairment of some rate. This approach focuses on the "average" organism or on some other particular level of effect, and suppresses the complicating factor of variation among individual organisms. This is a desirable feature in applied work. There are classical models which consider the spectrum of dose-effect relationships in any group of organisms but such detailed consideration would be a hindrance in generalizing the approach to consider many toxicants acting together.

G.3 Experimental support for additive action of lethal effects

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Summary. There are now many studies showing near-additivity of lethal effects in diverse mixtures. Dutch work with mixtures of 8 to 50 chemicals showed (a) additivity when each was present at only 1/50 of its LC50, (b) some contribution at only 1/400 of LC50, and (c) near-additivity for a variety of organic chemicals including pesticides. The U.S. EPA tested 175 pairs of toxicants and they showed mostly additive or somewhat infra-additive toxicity. In Europe, 30 effluents and polluted river waters caused lethality at a median value of 1.2 toxic units, i.e. slightly infra-additive. In Canada, oil drilling fluids with 5 to 9 components did not differ significantly from additive toxic action. Eleven effluents from debarking, kraft pulping, caustic extraction and mechanical pulping indicated additivity, since toxicity averaged 1.03 times the value predicted from chemical analysis. For 130 samples of pulp mill effluents, the additive LC50 predicted from chemical analysis was within 30% of the actual LC50 in 73% of the cases.

G.3.1 Complex mixtures studied in the Netherlands and U.S.A.

Working at concentrations in the lethal region, Könemann (1981) reported that each of 50 similar-acting organic chemicals contributed fully to lethality of the mixture, when each chemical was present at only 2% of its own lethal concentration. In other words, **small amounts of similar-acting chemicals were exactly additive in lethal effect.**

Similar results were obtained by Deneer et al. (1988); concentrations as low as 1/400 of the LC50s contributed to the joint lethal toxicity of 50 organic chemicals in mixtures. Other tests showed near-additivity of lethality to guppies, for a variety of 8 to 24 organic chemicals including pesticides, with several different actions. Concentrations as low as one-tenth of the lethal level appeared to contribute to the joint action (Hermens and Leeuwangh 1982).

A large number of lethal tests were sponsored by U.S. EPA and reported by Broderius (1991). In all, 175 isobole diagrams (e.g. Fig. G.1) were assessed for joint toxicity of two substances. Most of them showed additive toxicity or somewhat infra-additive (Fig. G.1). Broderius reported that a "complex type of joint action ... was observed in only a few of the ... tests using industrial organic chemicals. ... In only a few instances has a markedly more-than-strictly-additive type joint action been observed in our binary mixtures of industrial organics."

Another conclusion from the testing program of Broderius (1991) was that the toxicity of chemicals from the Narcosis I group was similar for different species of fish and daphnids.

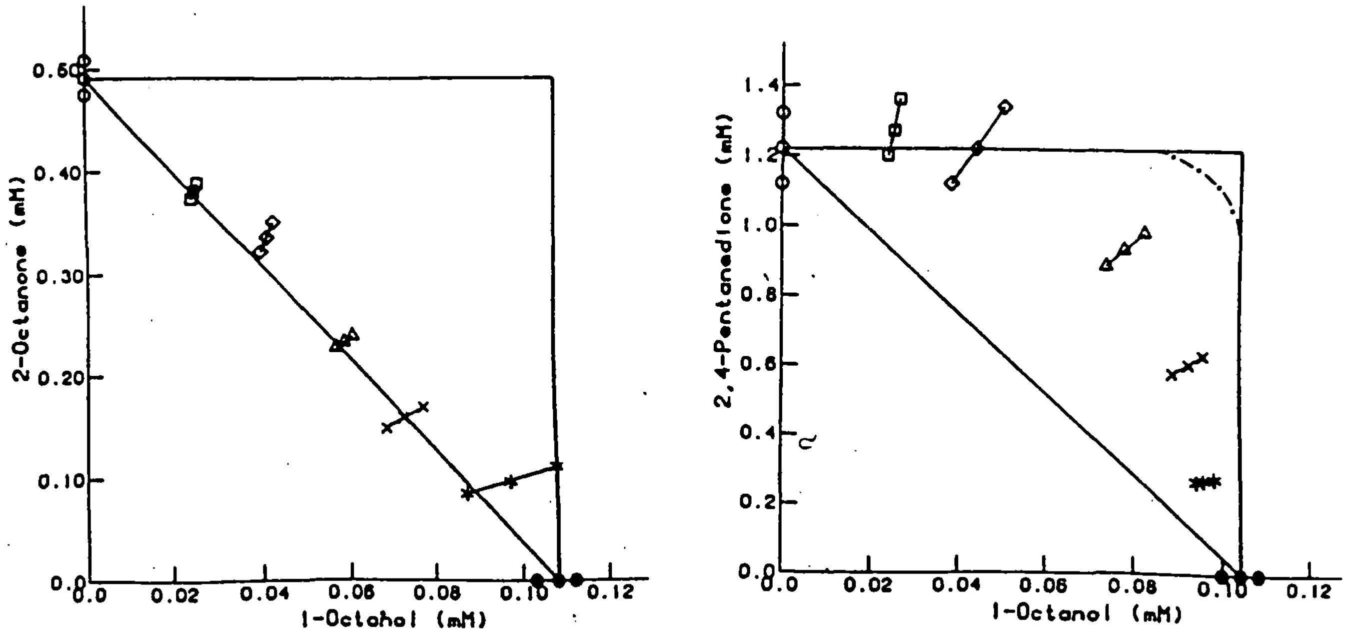

Fig. G.1. Examples of experimental results for toxicity of mixtures of pairs of organic chemicals. The diagram on the left indicates joint action that is essentially additive. The diagram on the right shows infra-additive joint action and also no addition at the top of the diagram. From Broderius (1991).

G.3.2 *Other supportive evidence*

Sprague and Logan (1979) tested three used drilling fluids (wastes from oil exploration), and found that lethality to fish appeared to be approximately additive for the 5 to 9 components of the fluids. The observed toxicities were 0.8, 1.2 and 1.4 times the predicted toxicities, none of those values significantly different from 1.0 which would represent additivity.

Information available in 1986 on joint toxicity of components of effluents and polluted river waters was summarized by EIFAC (1987) and is shown in Fig. G.2. For 30 tests with those complex mixtures, the median value for sum of toxic units at the LC50 was 0.85, very close to the value of unity which would represent additivity. Almost all of the values were between 0.5 and 1.5 toxic units (the points in Fig. G.2).

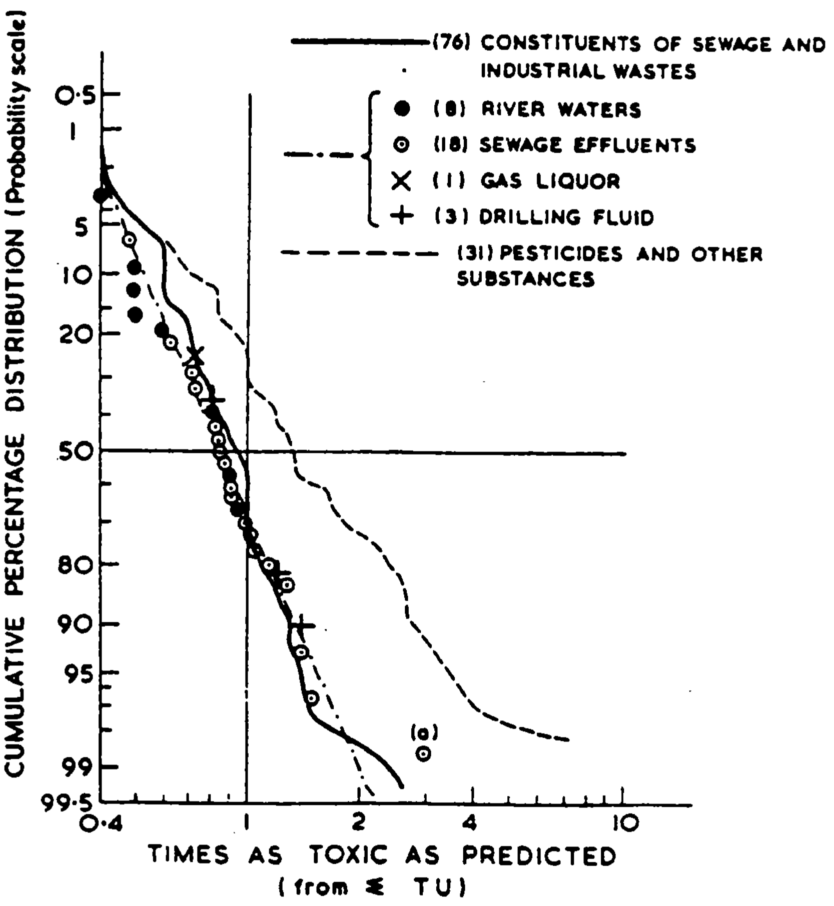

Fig. G.2. Compilation of acute lethality of complex effluents and polluted river waters, compared to the predicted toxicity based on additive effects of the individual components of those waters. The horizontal axis represents the sum of toxic units. The points with various symbols and the fitted dot-and-dash line represent the effluents and river waters. The solid line represents many experimental combinations of particular chemicals, also suggesting additivity, while the dashed line suggests more-than-additive action of pesticides. From EIFAC (1987).

Various mixtures of ten chlorinated organics showed agreement with the toxic units model in their lethal action, according to recent work by McCarty et al. (1992). The uptake of the substances was also followed, and the progress of bioaccumulation agreed with the degree of observed toxicity. The concept of an effective total body burden of the similarly-acting toxicants was also in agreement with the observed additivity of toxic action.

G.3.3 Near-additivity in Canadian pulp mill wastes

Some major projects carried out at B.C. Research in the 1970s showed remarkably good prediction of toxicity from chemical measurements of the components of pulp mill effluent. In eleven effluents from debarking, kraft pulping, caustic extraction and mechanical pulping, there was no clear evidence that the toxicity of the mixture departed from simple additivity of the component activity. On the average, measured toxicity to coho salmon was 1.03 times that predicted from chemical analysis, and the range of values for that calculation was 0.81 to 1.54, generally within the range of experimental error of the toxicity and chemical measurements (Leach and Thakore 1977).

Another large study by B.C. Research involved 130 samples of effluents from various kinds of pulp mills, some with secondary waste treatment and some without (Leach and Chung 1981). Nineteen of the chemicals thought to be toxicologically important (Appendix D, Table D.1) were measured by adsorption onto a resin column followed by gas chromatography. The chemical procedures took about 1.5 hours. Measured values of the nineteen substances were summed by toxic units to yield a predicted LC50 (Fig. 3).

For 73% of the samples shown in Fig. 3, the predicted LC50 was within 30% of the actual LC50 measured by toxicity test. The chemical prediction under-estimated the toxicity somewhat, but Fig. 3 shows that the results were generally in remarkably good agreement.

This work from B.C. Research does not appear to have been further developed. In particular, the next step of predicting sublethal toxicity has not been carried out. That sublethal work would be feasible today with the new short-term sublethal tests based on growth of larval fish and reproduction of daphnid crustaceans.

G.4 Other models for joint toxicity

There are many approaches. Most of them are not explained here since the application to water quality guidelines appears limited. Some of the common drawbacks are that they have been applied only to lethality, or have been developed for binary mixtures rather than multi-toxicant mixtures.

There are good reviews, and the various methods are given in Hermens et al. (1984a, b) and EIFAC (1987). More recent reviews centering on aquatic ecosystems are Christensen and Chen (1991) and Broderius (1991), the latter extensively cited in sections below. Also very comprehensive and useful in the field of mammalian toxicology is the review by Foulon (no date).

Some of the other techniques can be listed here. A formula that is somewhat more complex than that for toxic units, and more useful in theoretical or experimental work, is the *Mixture Toxicity Index* of Könemann (1981). Another approach is the multiplicative model, frequently encountered in fields other than aquatic toxicology; it is essentially a matter of multiplying together the *probabilities* of effects according to the strengths of individual toxicants in a mixture. It does not appear to be of primary interest in aquatic studies (Parrott and Sprague in press).

Of more interest are isobole diagrams, similar to Fig. 2 but with tests of a wide variety of combinations of the two toxicants. Such diagrams allow a display of the degree to which toxicants join in their effects, so that the type of action can be easily interpreted and displayed (infra-additive, additive, etc.). The diagrams are limited to binary mixtures, or conceivably three substances together in a 3-dimensional diagram. There is an algebraic way to assess more than three toxicants using the general isobole approach (described in Foulon no date), but it appears to be a particular case of the toxic units method.

An approach used for polycyclic aromatic hydrocarbons (PAHs) is to use a dominant component as the model for the whole group of chemically similar compounds, and assume that they all act in the same way and with the same potency. This is conservative, and useful with such dangerous substances as PAHs when human health is in question.

G.5 Sublethal action of mixtures

Summary. There are a few sublethal experiments indicating that there may be no sublethally "safe" concentration of a chemical when it is part of a mixture of substances in the receiving water. Tests of 5 to 25 substances of similar action indicated that they contributed at least half of their individual sublethal toxicities to the overall effect of the mixture. Even for a combination of 14 substances having several modes of action, each chemical apparently contributed about half of its toxicity to the action of the mixture.

The essence of recent findings is that assessments of water quality should indeed be concerned about joint sublethal action of chemicals, whether they are similar in action or dissimilar. One reason for concern is that there may be no such thing as a no-effect or "safe" level of some toxicants, when they are part of a mixture of toxicants in the receiving water. Action at the sublethal level is of primary interest when considering water quality guidelines, and the work on that topic is important although small in amount. Rapid sublethal tests have been used in research to evaluate combined action.

One early finding was a 70% reduction in photosynthetic rate of algae when exposed to a mixture of 10 metals, each at its own water quality objective as set for the Great Lakes (Wong et al. 1978). Photosynthesis was reduced by 40% when each of the 10 metals was at one-tenth of its water quality objective. Various interpretations could be made, including early objectives that had been set too high, as well as joint toxic action by the metals.

Dutch workers tested mixtures of 5 to 25 organic substances with similar action, for effect on reproduction of *Daphnia* (Hermens et al. 1984a). Toxicity was assessed by the toxic units method (above), i.e. summing fractions of the individual effective concentrations. Mixtures were effective at 2 SLTU or less, that is, the **individual substances appeared to retain at least half of their potencies in the mixtures.** Furthermore, the joint action in the mixture of 25 substances appeared to be stronger (1.5 SLTU, i.e. retaining two-thirds of individual toxicity) than in the mixture of 5 substances (2 SLTU), even though each substance was present at only about 1/17 of its individual effective concentration.

This work suggests that **there may be no such thing as a sublethally "safe" level (NOEC) of an individual substance when it is part of a mixture of substances with similar action.** That conclusion has been reached, not only here and by the Dutch researchers who did the work, but by Christensen and Chen (1991) as the result of extensive review [12].

Comparable results were obtained for mixtures embracing different modes of action. A mixture of 14 substances having several types of chemical structures and modes of action affected *Daphnia* reproduction at 1.9 SLTU (Hermens et al. 1984b). In other words each of the **dissimilar toxicants apparently contributed about half of its toxicity to the action of the mixture**, when each was present at about 1/7 of its individual effective concentration. For ordinary organic chemicals (Narcosis I type) the addition of effects was nearly exact in experiments on Daphnia reproduction with mixtures of 10 and 25 chemicals, i.e. at concentrations which were only 4% of the individually effective one (De Wolf et al. 1988).

The results described in the previous paragraphs are from relatively few experiments, and indeed, some other experiments in the literature show a variety of results including lack of combined action [13]. Still, the findings above suggest that in addition to approaches derived from single-chemical guidelines, some method or methods of evaluating multiple toxicants in surface waters is desirable.

The conclusion was stated as follows by Broderius (1991).

> **"no-effect levels of separate chemicals may have little meaning for mixtures ..."**

It should be feasible to apply techniques for mixture toxicity to BPME. Despite the large number of compounds present in such waste, the active substances may represent a manageable number. The work of Leach (1991) focused on 30 chemicals that were considered to contribute most of the toxicity. Suntio et al. (1988), on the basis of a major review of technical data, and a background of chemical and toxicological knowledge, offer the following opinion.

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[12] Christensen and Chen (1991) phrased it this way: "... it would, therefore, appear that there is no threshold under which a chemical does not contribute to the mixture toxicity."

[13] Some experiments have shown that sublethal effects were far from additive, and this may be especially true for metals. Spehar et al. (1978) found antagonistic action of cadmium and zinc in effects on growth of flagfish (although reproductive effects were additive). Eaton (1973) concluded that the combined effect of copper, zinc and cadmium on growth and reproduction of fathead minnows was similar to the effect of zinc alone.

> **"We suspect that, in the case of pulp and paper effluents, the toxic effect may be controlled by the sum of the effects of perhaps 50 chemicals."**

The experimental work cited in this section and the preceding one, dealt with up to as many as 25 chemicals acting sublethally, and up to about 50 acting lethally. The principles derived from that work may apply to pulp mill effluent, and should be capable of dealing with an assessment of the active chemicals in this type of effluent.

G.6 Advice from experts

Summary. Most of the experts interviewed did not offer advice on developing guidelines for mixtures of pollutants. One suggested caution and one was in general agreement with recent research findings.

The topic of mixture toxicity was not mentioned by most of the people interviewed. Only a couple of people commented, and one, Dr. Hodson of the Dept. of Fisheries and Oceans, pointed out the difficulties and deficiencies of predicting mixture toxicity. He noted that we do not have a great deal of experience upon which to base any system of estimating joint action. There is almost nothing in the way of field work that has been deliberately designed to evaluate action of mixtures.

Richard Lloyd of United Kingdom provided a chapter on complex effluents (Lloyd 1991b) which summarized experience on the nature of mixture toxicity. At the sublethal level, he considered that there simply was not much information, and relied on his own experience with effluents. For effluents containing different *types* of toxicants, i.e. acting by different mechanisms, he concluded that at higher concentrations, near their levels of sublethally harmful effect, such diverse toxicants tended to be additive in contributing to the total effect of the mixture. At very low concentrations of individual substances, harmless in themselves when acting separately, Lloyd judged that they did not usually contribute to the toxicity of the mixture, a conclusion which disagrees with the findings of Hermens et al. (1984b) cited above. For a mixture of toxicants with similar action, Lloyd's analysis agreed with that shown by the Dutch research.

G.7 Guidance from industrial hygiene and other fields?

A review of the approaches taken by most regulatory agencies indicates that some form of additive model is generally applied to the problem of mixtures.

Denise Foulon (no date).

Summary. Many government or professional organizations evaluating hazards of airborne (or other) contaminants in the work-place recommend a simple toxic units approach based on additivity, to predict effects of a mixture. This is especially true for similar-acting toxicants. Among the agencies are the American Conference of Governmental Industrial Hygienists, the United Kingdom Chemical Industries Association, the Netherlands Government Institute for Public Health and Environmental Protection, a (former) U.S.S.R. health agency, and the World Health Organization. More complex approaches emphasizing case-by-case risk assessment are used by some other agencies such as the U.S. Environmental Protection Agency, notably at hazardous waste sites.

Are there better tools for assessing multiple toxicity, if we look in other areas of toxicology? It has often been an avenue of conceptual advancement in the past, to look outside the field of aquatic studies and borrow techniques from pharmacology or some other applied discipline. One example has already been given -- in industrial hygiene it is standard practice to assume additivity of the actions of several toxic substances if they have the same or similar mode of action (ACGIH 1988). For toxic substances with unlike actions, the industrial hygienists assume independent action, in other words the concentration of each chemical is compared to its individual guideline.

Aside from that application in industrial hygiene, aquatic toxicologists may be as advanced as anyone in concepts of joint toxicity that might be useful for developing guidelines. In a review of other fields a few years ago, Parrott and Sprague (in press) were surprised that pest control workers, and horticulturists concerned with air pollution, did not have more advantageous techniques for estimating thresholds of mixture effect. Much of the work used isobole diagrams, multiplicative models, or simple methods, as discussed in section G.4. One horticultural paper stated that the isobole approach was "not amenable to detecting thresholds". The models were used with subjective interpretation, to judge the degree of effect of two substances acting together, and the category of their joint action. However most methods were not useful for assessing mixtures of many substances, and as mentioned they were not useful for establishing thresholds of effect, as would be desirable for developing guidelines.

The conclusion that other fields of endeavour do not have advanced techniques to offer, is supported by a thorough review by Foulon (no date). The reviewer was interested in techniques for hazardous waste sites, and covered the public health field in her search. Many of the authors listed in her bibliography are different from the ones writing in aquatic toxicology, but the techniques considered and used, the terminology arising from an older generation of statisticians and toxicologists, and the general conclusions about useful approaches, are all very similar to the status of thinking among scientists working in aquatic toxicology.

One of the groups that Foulon covered in her review was the American Conference of Governmental Industrial Hygienists, already discussed above (ACGIH 1988), and she found that they continued to use the same approach in 1990 - 91. In the United Kingdom, the Chemical Industries Association uses the same toxic units approach for airborne toxic substances that are not known to have actions that are independent, infra-additive or supra-additive (CIA 1985). The Dutch Government Institute for Public Health and Environmental Protection uses the additive approach; Foulon quotes a statement that it is "assumed in all cases that chemicals have additive effects if the mechanism is the same" (Linders 1990). Foulon reports that regulatory agencies in the (former) U.S.S.R. adopted a simple additive model for calculating occupational health standards for multiple air contaminants, based on fractions of the individual maximum permissable concentrations.

The Canadian Contaminated Sites Remediation Program emphasizes case-by-case risk assessment. That is also done by the U.S. Environmental Protection Agency, which describes a complex approach, arising from the substances involved, their actions, and the availability of data (Foulon no date). Several techniques are recommended in the U.S.A., but among them is an assumption of a toxic units model based on addition of doses, in cases where there is little known about the interaction among components of the mixture (U.S. EPA 1986a) [14]. This procedure is for non-carcinogenic substances that have the same mechanism of toxicity. Even more complex and specialized are approaches for U.S. hazardous waste sites used by the "Superfund" administration and the Air and Waste Management Association. Those procedures will not be reviewed here except to say that minimal use is made of additive models for mixtures. Finally in the U.S., the National Research Council sets forth a number of methods for testing and analyzing complex mixtures, with an assumption of additivity as the basis of some (U.S. NRC 1988). NRC suggests that when there are no data available for the toxicity of the whole mixture, that can be approximated by summing the known toxicities of the components.

At a more international level, the World Health Organization recommends the simple toxic units approach for predicting effects of a mixture of similar-acting toxicants, and in particular for evaluating the hazard of airborne contaminants in the work-place (WHO 1978, 1981). It was mentioned at the beginning of the appendix that EIFAC (1987) proposed this method of assessing joint effects at high concentration.

G.8 Recent theoretical review of joint toxicity

The complete field of mixture toxicity to aquatic organisms, and how to test and analyze it, is examined from advanced statistical and theoretical viewpoints by Christensen and Chen (1991). They consider sublethal effects near the end of their review, as well as practical application of the findings to water quality objectives. In view of the sophistication of these authors as statisticians and modellers, and their long-continued study of mixtures, it is of some importance that they finally conclude that a simple *toxic unit* model is sufficiently accurate in most

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[14] Foulon quotes U.S. EPA that "several studies have demonstrated that dose additive models often predict reasonably well the toxicities of mixtures composed of a substantial variety of both similar and dissimilar compounds".

circumstances to be used for prediction and in fact for regulating discharges. Their exact wording is as follows, in their section on Water Quality Criteria [Guidelines].

> "In the absence of calibration data, we believe that the water quality criterion, [given as an equation], based on concentration addition, is appropriate. It is more protective for aquatic life against adverse effects from chemicals in a mixture than individual limits for toxicants based on no addition, and because near-concentration addition is so common, it is also more generally applicable."

When Christensen and Chen (1991) speak of the "absence of calibration data" they mean a lack of empirical measurements of the toxicity of the mixture of concern. Concentration addition is another description of the toxic units model in this case, and that model is the one shown in their Equation 64.

APPENDIX H
APPROACHES USED ELSEWHERE, PARTICULARLY BIOLOGICAL APPROACHES

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Water quality guidelines are purely scientific in their attributes, but they are the basis for part of a system for controlling water pollution. Aquatic organisms live in rivers, lakes, and seas, not in effluents, and require good quality surface water. Water quality guidelines define the quality required in surface waters, and may be used to develop *water quality objectives* for bodies of receiving water. Or, in turn, *regulatory standards* may be set. Such limits in the receiving water constitute the only strong predictive tool for evaluating in advance of an anticipated waste discharge, whether or not that discharge is likely to cause harmful ecological effects in the body of water (i.e. tactic no. 2 of a general control strategy, Appendix B).

Thus, guidelines are the scientific base for part of the overall control process, and should provide the scientific base for technically valid water quality numbers, narrative descriptions, *or other methods such as testing that will yield data for decisions* on water quality. This appendix attempts to provide some examples of biological approaches and techniques that might be considered for future guideline development.

This appendix deals mostly with three examples of control or management techniques, partly because it is easier to describe the control applications and give examples, than to frame the topics as purely scientific water quality guidelines. Nevertheless, the techniques might be adapted for establishing Canadian water quality guidelines which were relevant to discharges of BPME. Further details of the control and management approaches are given in Sprague (1990b, 1991) and Sprague et al. (1991).

H.1 Water quality limits based on sublethal toxicity testing in U.S.A. and Australia

Summary. Permits for industrial discharges in U.S.A. now require sublethal testing, with a goal of no sublethal effects in the receiving water. This is in addition to chemical water quality objectives. Australia has drafted the same general requirement for waters receiving pulp mill wastes. The implied national guideline consists of stipulating the method of testing and the endpoint of no sublethal effect in the receiving water.

The U.S. now has an excellent regulatory approach for effluents which includes each of the **three tactics** in the strategy outlined in Appendix B [15]. Toxicity testing is done in both tactics no. 1 (best technology) and no. 2 (water quality limits). Chemical variables are also specified. Ecological monitoring is also required (tactic no. 3). It is the combination of approaches that provides comprehensive environmental protection, but the instream water quality limits are of chief interest here.

The U.S. EPA uses sublethal testing to establish a water quality standard, and to assess compliance with the standard (Hanmer 1991, U.S. EPA 1991). **The water quality objective,** stated simply, is that there should be no sublethal effects on (selected) aquatic organisms in the receiving water, beyond the boundaries of a (defined) mixing zone [16]. That objective is, of course, site-specific since it depends on the dilution available, the mixing characteristics of the waterbody, and the way the mixing zone is designated.

The water quality guideline implied by this approach consists of (a) the toxicity test methods that are specified, (b) the endpoints specified for the tests, and (c) a statement that the endpoints are not to be exceeded in the receiving water.

To carry out this approach, sublethal tests are performed on the effluent of concern. The endpoints of the tests (e.g., no-effect concentrations) are estimated and compared with the concentrations that are calculated to prevail at the edge of the mixing zone. Low flow conditions in the river are customarily used.

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[15] The U.S. EPA speaks of a "triad" approach, and it is similar to but less comprehensive than, the three-tactic strategy outlined in Appendix B. The third item of the U.S. triad is biological assessment, the same as described here. EPA splits up tactic no. 2 into chemical-specific and whole-effluent water quality limits. They do not include best-available-treatment limitations as one of the items in the triad although that tactic is most definitely part of their control strategy.

[16] Such a mixing zone must be allowed, either by formal specification or by unwritten agreement. If there were no mixing zone, then the limit would apply at the end of the effluent pipe, and indeed would apply to the effluent itself. Clearly, water quality guidelines, objectives, and standards are intended to apply in the surface water, not the effluent. If the effluent itself met surface water quality requirements, presumably there would be no need to discharge it; the discharge could simply be used as raw water by the industry.

This procedure can be used in a predictive mode, i.e. as a water quality objective following tactic no. 2. That is done by measuring and/or calculating the minimum dilution available at the edge of the mixing zone. Knowing that, the maximum degree of toxicity can be calculated for the effluent, a variable that can be used ahead of time for design purposes if the effluent toxicity can be predicted or is generally known from other locations. Once the industry is operating, the same testing procedure is continued, to monitor compliance with the toxicity objective.

This approach has been used in the U.S. since 1984, but does not yet appear to be standard anywhere in Canada. Most Canadian regulators do not seem to be familiar with the concept. It should be used in control programs, and the general principle would be suitable as a separate **Canadian water quality guideline** (section 4).

H.1.1 Details of sublethal toxicity tests for U.S. water quality objectives

Further details of the U.S. sublethal testing are as follows.

> Three species are tested, ordinarily, to derive NOECs:
> (a) 7-day growth and survival of newly-hatched fathead minnows;
> (b) 7-day survival and number of young in the small crustacean *Ceriodaphnia*; and
> (c) growth/reproduction during 4 days for an alga (*Selenastrum capricornutum*).
>
> In rivers, dilution is for low flow, normally the 10-y minimum for 7-d average flow (7Q10).
>
> The mixing zone might be one third of the width of a river.
>
> Edge-of-mixing-zone calculations are made as toxic units (lethal or sublethal, section G.2) to simplify arithmetic. The limit is a maximum 4-day average (U.S. EPA 1991).

For regulatory purposes in the U.S.A., no safety factor is used, except that the NOEC is applied at the edge of a mixing zone that has been decided upon; further dilution would in effect act as a safety factor. The expected concentration at the edge of that zone is already known from studies in the receiving water and modelling for times of low flow. A comparison is made between the expected concentration at the edge of the mixing zone, and the NOEC determined in the toxicity tests. The NOEC must be higher than the average concentration expected at the edge of the mixing zone, i.e. conditions must be "safe" outside the mixing zone.

The U.S. regulatory program with sublethal toxicity tests is well designed for protecting aquatic ecosystems from toxic substances in effluents. Testing three different types of organisms is protective, since there are major differences among different kinds of plants and animals, in relative sensitivity to various kinds of effluents. Addition of a non-algal micro-organism to the battery of tests might be even more revealing since they are often most sensitive (Slooff et al. 1983).

H.1.2 Proposed Australian kraft mill regulations

The limits proposed in 1989 for Australian bleached kraft mills may be the world's most comprehensive, rational and brief set of controls for such effluents (described in Sprague 1990b). They provided a balanced blend of three-tactic limits. Testing was both chemical and biological. Sublethal tests were included for setting limits at the edge of a mixing zone, i.e. as water quality objectives, in similar fashion to the description above for use of these tests in the U.S.A. The proposed Australian regulations have not been applied to any mill, partly because they must be adopted by individual states and partly because no new mill has been built since 1989.

H.2 Use of biotic indices in U.K. for river classification and quality objectives

Summary. Biotic indices of the quality of river invertebrates will be used in Britain to classify sections of rivers, and to set numerical goals (water quality objectives), in parallel with chemical measurements and objectives. In case of disagreement of classification, there is a procedure for "biological over-ride".

A major development in the use of biological survey is a British proposal to use **numerical indices of quality of biological communities as environmental quality objectives**, in addition to use of the indices for classifying rivers. Although this may not be a technique for early application to Canadian waters receiving pulp mill effluents, it illustrates the changes in approaches that are occurring, and particularly, it is part of a shift to biological approaches. Further details are given in Sprague (1992).

As the result of recent legislation (Water Act in 1989, and Water Resources Act in 1991) the National Rivers Authority (NRA) is in charge of river water quality for England and Wales (NRA 1991). The procedures described here were published by the British agency in order to obtain comments by interested parties, but appear to be proceeding towards implementation.

(1) Rivers and sections of rivers are first classified according to water quality, existing and desired.

(2) Water quality objectives are then set by reference to the above classification.

(3) Decisions are then made on how much of certain kinds of pollutants can be discharged, what should be reduced, and which sources should be cut back.

Biological surveys of benthic invertebrates are used to obtain a biotic index of water quality, and the index then plays a major role in three aspects of water quality management. Biology is used in the initial classification of the sections of stream (item 1 above). The value of the biotic index is used as a water quality objective for a section of river. (item 2 above). Finally, the index obtained by monitoring surveys is used as a means of checking compliance with the objectives.

H.2.1 The British classification scheme

In the past the classification has been fairly simple, with Classes 1, 2, 3 and 4 representing water quality that was "Good, Fair, Poor, and Bad" respectively. Now there will be more formal categories and objectives.

> **Categories of use** will include salmonid fishery, cyprinid fishery, water sports, and water supply. Each use will have attached to it, a specific set of **chemical standards**, and potentially, **biological standards**.
>
> **The general classification scheme** will continue to have 4 or 5 categories, used to describe existing quality, and also as targets for quality. Each category would have specified values of key chemical parameters (DO, BOD, ammonia) and biotic index (Table 1, page 18+, NRA 1991). Standards have been issued for drinking water sources and bathing waters. Others have been proposed for various categories of fish and other uses (Table A2.1, p. 60, NRA 1991).

H.2.2 Biotic classification and objectives

Britain has done biological surveys of all rivers systematically every 5 years since 1970. Biotic Indices have been developed, by using a numerical scoring system to represent the kinds and numbers of organisms present, and their sensitivity to the usual kind(s) of pollution. The indices have become streamlined, simplified, and easy to use. They are robust and accurate in assessing many kinds of pollution and environmental degradation [17].

The system is used by carrying out the following steps.

(a) Water quality is judged from a biological survey of the macro-invertebrates, using one or more designated biotic indices (they are similar to each other).

(b) The biotic index (score) for a community is compared with what would be expected if the location were completely free of pollution. This is a new development and is based on accumulated knowledge over the years. Various physico-chemical measurements (gradient, velocity, substrate, mineral content of water, etc.) have been associated with type of biological community and put into a computer model (RIVPACS). The result is an Environmental Quality Index ...

$$\mathrm{EQI} = \frac{\text{expected status}}{\text{observed status}}$$

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[17] British biotic indices, slightly adapted for North America, have been used in surveys of river pollution by classes of students at the University of Guelph. The indices worked very well, and were superior to some other types of indices and methods of comparing communities. The biotic indices tended to reflect *water quality* as opposed to other types of habitat degradation. There are biotic indices developed in North America, but they are not in common use yet.

(c) Certain chemical standards are also used to classify a section of river. The chemical and biotic categorizations are **both** used.

(d) Values of EQI are associated with the different levels of classification (Class 1, "Good"; Class 2, "Fair"; etc.).

What if the biotic and chemical categorization disagree? In another new development, a **"biological over-ride"** has been installed in the procedure. The biotic information would take precedence in classifying the section of river. When the biological over-ride was used on one collection of information from rivers, it changed the classification of about one third of the lengths of rivers. The overwhelming net effect, was to upgrade sections of river compared to their classification based on chemical water quality objectives (Table A3.6, p. 76, NRA 1991).

Water quality objectives grow out of this approach simply, by using the biotic scores or EQI as objectives. The British intend to do that. A value of EQI would be set for a stretch of river as a regulatory objective or goal, in parallel with chemical water quality objectives.

H.3 Swedish characterisation of industrial discharges

Summary. Sweden has published a tiered approach for "Characterization of Industrial Discharges" (CID), i.e. judging their potential harmfulness. The four components of the approach are toxicity, degradability, bioaccumulation, and chemical nature. Toxicity is first judged by tests of acute lethality, and if receiving-water concentrations will be >0.1 LC50, the discharge is considered acutely toxic and remedial actions should be undertaken. If the effluent will be at concentrations >0.01 LC50, it is considered "capable of causing damage", and investigation should proceed with sublethal tests of Tier 2. In effect, the results of biological tests, with safety factors, are being designated as water quality objectives.

The third example of biological testing is a formal procedure for **"Characterisation of Industrial Discharges"** (CID) used in Sweden. A tiered approach with toxicity tests and chemical measurements is used to evaluate the harmfulness of effluents, before allowing discharge. Although the techniques do not develop guidelines, which is the topic of this report to Environment Canada, they provide another example of direct use of biological tests. This Swedish CID system is described in section H.3.

This approach is used and recommended by the Swedish Environmental Protection Agency (SNV 1990) has been published as a handbook. Although the approach is neither novel nor radical, it is briefly described here because reasonable ways of assessing an effluent have been put together in a convenient form.

An effluent is characterized under four topics: *toxicity; degradability; bioaccumulation; and chemical nature.* The last three items are largely diagnosed by chemical analysis. Degradation is followed by oxygen consumption during decomposition, or by the disappearance of organically-bound carbon. Bioaccumulation is largely predicted from measurement of the octanol-water partitioning coefficient, or by direct experimentation with organisms if necessary. The chemical nature is determined by conventional (but advanced) procedures of analysis.

The toxicity tests are standard ones, arranged like the rest of the approach into tiers of increasing sophistication. If a potential problem is apparent from the relatively simple tests of Tier 1, then remedial action is taken or else the investigation proceeds to more sensitive tests of Tier 2.

Tier-1 tests are ordinary acute lethal tests with fish, crustacean, alga, or other proven organism. **Tier-2** tests are reproductive evaluations with invertebrates, early-life-stage tests with fish, physiological changes in trout, or long-term survival of mussels and algae. **Tier-3** tests are even more subtle ones such as delayed effects on fish, physiological effects in caged or wild fish from the actual receiving water, and model ecosystem evaluations.

Useful to the present report are some rules of thumb to assist in deciding whether to proceed into another level of testing. If the concentration of the waste in the receiving water, after "primary dilution", was expected to be **greater than 0.1 of the LC50** of Tier-1 tests, the discharge should be considered acutely toxic. Remedial action should be taken.

If, after primary dilution, the effluent was expected to be at concentrations **greater than 0.01 of the LC50** from Tier 1, it would be considered "capable of causing damage". The test program should proceed with Tier 2.

The **bio-tests are being used as *de facto* water quality objectives.** If the concentration in the receiving water is > 0.01 LC50, then the waste is considered potentially harmful [18]. The method could be defined in suitable words, to make it a water quality guideline.

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[18] For Tier-1 tests with invertebrates, results would be given as EC50 rather than LC50. The Swedish document is careful to say that concentrations lower than 0.01 LC50 should not be taken as proven "safe" for aquatic organisms. Their statement was, as noted, that concentrations higher then 0.01 LC50 were potentially harmful.

APPENDIX I
SUBLETHAL TEST + SAFETY FACTOR = GUIDELINE?

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I.1 Fundamentals

Summary. The only way to measure toxicity is with a biological test.

Toxicity can be measured only by using a living organism or living system. Toxicity cannot be measured by chemical tests; they can only predict the outcome of a toxicity test. If it is desired to find out if something is toxic, say an effluent, a biological test should be used. If that effluent is indeed toxic, and it is desired to find out what substance or substances are responsible, biology cannot uncover that, so chemical analysis should be used. The two approaches should be used together in a complementary manner.

I.2 Toxicity as a water quality guideline

Summary. A sublethal test or tests should be done with the effluent itself. The defined method of testing, defined threshold-effect endpoint, and a safety factor, would constitute the fixed water quality guideline, i.e. the method of determining the maximum concentration considered safe in the receiving water. Applying this guideline at any given time or place, the concentration obtained would be a local water quality objective. Calculations from the known available dilution would estimate whether the toxicity of the effluent was low enough to meet the objective in the receiving water.

It follows from the preceding section, that toxicity is a logical and desirable feature to use as a guideline. Water quality guidelines are intended to provide values that would protect aquatic organisms from sublethal effects. Sublethal toxicity tests measure that characteristic directly. This appendix considers using toxicity as a guideline, and outlines suitable test methods.

An appropriate procedure would use a sublethal test or tests on dilutions of the effluent, to estimate the lowest-observed-effect concentration (LOEC), threshold-effect concentration (TEC), or other sublethal endpoint considered satisfactory. The testing would be done on the effluent itself in order to get a strong response that could be related to concentration. A safety factor would be applied to the endpoint, if it had been decided to incorporate one into the method (see section I.4). The intent would be to obtain a concentration of effluent that would be protective of all species of aquatic organisms, as is the case with water quality guidelines stated in terms of concentration of a chemical.

This toxicity **testing procedure would be the water quality guideline** for toxicity. Since each step would be specified according to a standard method, the procedure would be fixed and uniform for all locations.

To apply this guideline for control purposes in a given situation with an existing discharge, the toxicity test on effluent would be run as specified. The method would yield a concentration of that particular effluent at that time, and that concentration would be the site-specific water quality objective applicable at the time. It would be stated as a maximum concentration of the effluent in the receiving water. A calculation could then be made on the basis of previous determinations of mixing and dilution, to evaluate whether or not the actual concentration in the receiving water would exceed the water quality objective. That would be for monitoring purposes, to see if the effluent met the water quality objective.

The system could also be used in a predictive mode for planning and considering proposed discharges, in a similar way to chemical water quality objectives (water quality limits, tactic no. 2 of Appendix B). Having determined the likely mixing pattern and dilution available, the objective of no sublethal toxicity in the receiving water could be calculated back to a maximum degree of toxicity that would be acceptable in the effluent. That could be used as a design parameter.

This is essentially the method of U.S. EPA (Appendix H) for a standard water quality objective which can be paraphrased as "no sublethal toxicity at the edge of a defined mixing zone".

Using sublethal **tests in the receiving water** (e.g. caged fish in a river) is not recommended because the effluent would normally be greatly diluted, and weak responses of the test organisms would be difficult to detect. Also there would be little control over the concentration tested, because of the vagaries of mixing. Such instream tests might be more suitable for monitoring of effects, i.e. tactic no. 3 of a control strategy (Appendix B), which would be the purpose of environmental effects monitoring in the pulp and paper effluent regulations.

I.2.1 Advantages and disadvantages

The advantages in using biological tests, according to OECD (1987), include the following.

(a) They give a direct measure of effect.
(b) They measure combined effects which could be found in an effluent.
(c) They evaluate components of the effluent for which there are no data on toxicity.

Some potential disadvantages can be listed.

(a) Some people are unfamiliar with the approach and the tests.
(b) A perception that chemical numbers are more "scientific".
(c) If there were no effect in a toxicity test, one could not determine how close the effect-level was -- the toxicity of the effluent might be 90% of that required to cause an effect, or it might be only 1% of the effect-level.
(d) If it was desired to check that a certain toxicity objective was being achieved in the receiving water, it would be difficult or impossible to do so directly, whereas direct chemical measurements could be made in the receiving water for many or most chemical objectives.

I.3 Types of sublethal toxicity tests

Summary. Short life-cycle tests with daphnids and algae, and early-life-stage tests with fish, are sensitive and recommended for toxicity guidelines for effluents. Biochemical tests such as induction of mixed-function oxygenases are sensitive for monitoring exposure but are not recommended for establishing guidelines at present, because of questions on their relation to whole-organism effects.

Types of sublethal aquatic toxicity tests are reviewed elsewhere (Sprague 1990a) and here they will simply be divided into whole-organism tests and within-organism studies.

Among whole-organism tests, recent activity centres on reproduction and growth in early-life-stage, partial life-cycle, and life-cycle tests with fish, daphnid crustaceans and algae. The time required for these tests has been greatly shortened over the past decade.

Among within-organism tests, there is currently much biochemical research, mostly intended to discover mechanisms of toxic action. These are briefly discussed here (section I.3.2), but are not recommended as primary tools for water quality guidelines.

I.3.1 Reproductive tests

> *Reproduction is the most sensitive, consistent, and relevant end point tested to date in the laboratory, in mesocosms and experimental streams, and in field situations near some pulping discharges.*
>
> J.W. Owens (1991)

Owens' quotation is taken from his definitive review of Scandinavian and other research on sublethal effects of pulp mill effluents. Owens (1991) concludes that "reproduction and early life stage impacts are ideal end points to assess toxicity and to discover toxic mechanisms." and points to agreement with earlier conclusions of Sprague (1978) and Kovacs (1986).

These tests with fish, invertebrates and algae have become, in the last two decades, the standard ways of providing data for water quality guidelines. Shorter life-cycle tests using smaller animals (*Ceriodaphnia*) are now used routinely, so that full reproductive tests can be done in 7 days. Algal and microbial tests are faster. Abbreviated tests on the early life-stages of fish (newly-hatched larvae) can be done in 4 to 7 days and since this stage is usually among the most sensitive of the entire life cycle, results are a good estimate of the long-term no-effect concentrations.

Environment Canada has now defined standard methods for the four rapid sublethal tests mentioned in the previous paragraph (EC 1992a, 1992b, 1992c, 1992d).

I.3.2 Biochemistry/physiology

A good example of these within-organism tests is the use of mixed-function oxygenases (MFO) as an index of exposure to pulp mill effluents. A suitable standard method has been promulgated in Canada (Hodson et al. 1991a) based on cytochrome P-450 induction (EROD is a commonly-measured P-450). MFO tests are providing guidance on apparent history of exposure of fish communities to polluted conditions.

As with many biochemical tests, the induction of MFO in fish must be interpreted with care. The Fisheries and Oceans document of Hodson et al. (1991a) gives the following good advice on interpretation.

> "The biological significance of P-450IA induction is not completely known."
>
> [measurements] "of MFO induction justify further studies of biological impacts. ... Induction is one of the easiest and most sensitive responses to detect. If induction can be avoided, presumably other ... biological responses will also be avoided. If induction is detected, more detailed studies are needed of the survival, growth, reproduction, and bioaccumulation of inducers by the local fish community. ... lack of induction does not mean 'no effect' ... Measurement of MFO induction signals only an increased probability of a suite of associated responses."

The conclusion is that MFO and some other biochemical/physiological assays can be useful indicators in a field monitoring program, but a definitive statement about damage should be based on a whole-organism effect. For use in connection with water quality guidelines, a test giving an unequivocal deleterious effect on the whole organism should be used.

I.4 Safety factors

Summary. A safety factor in the region of 0.1 is recommended. This would be applied to the threshold of concentration causing sublethal effect, as determined by test for any given pulp mill effluent. The safety factor would be intended to allow for sensitive species in the receiving community, and sensitive individuals and life-stages of those species. The toxicity water quality guideline would be defined by specifying the sublethal test(s) to be used, and the value of the safety factor.

A factor in the vicinity of 0.1 applied to the threshold for sublethal effect seems to represent the weak consensus that exists in aquatic toxicology, on the magnitude of the safety factor. That is judged by the value of 0.1 used by the CCME, and values approximating 0.1, suggested in two European guidebooks on managing effluent toxicity.

A safety factor would produce values that were well below the no-effect concentration in a particular test or tests. The safety factor would allow for possible inaccuracy of the test, for effects that might occur with longer exposures than were possible in the laboratory tests, for more sensitive individuals in a population, or for other more sensitive species in communities.

Estimation of safety factors is largely a matter of professional judgement based on experience. Such factors are more firmly established in matters of human health and might be borrowed from that source. The usual concepts of risk assessment (Bonsor et al. 1988) serve as a background for the adoption of safety factors.

I.4.1 Safety factors in aquatic toxicology

There is no universally recognized value for a safety factor for toxicity of water pollutants to aquatic organisms. Two European organizations provide "guide-book" reviews with approaches that are similar to safety factors, phrased in terms of dilution available. A Canadian safety factor of 10 can be used to derive a water quality guideline; it is described as 1/10 of the lowest chronic LOEL in the data on sublethal toxicity which are available for the toxic material being considered (CCME 1991).

A Canadian water quality guideline can also be derived from acute lethality (LC50) or other acute data (EC50). The "acute-chronic ratio" (ACR) is empirically determined as the ratio between the acute LC50 (or acute EC50) and the No-Observed-Effects Level (NOEL) for sublethal effect, in the same species. If the ACR cannot be determined empirically, there are standard "application factors" (the inverse of ACR) that may be used to move from lethal to sublethal ranges, the values being 1/20 for non-persistent toxicants and 1/100 for persistent toxicants. The ACR or application factor is applied to the lowest LC50/EC50 in a set of data, to estimate the lowest NOEL, and that is used as the water quality guideline. Since no safety factor is used, the procedure for acute data could obtain a different water quality guideline than might be obtained if chronic data were available, in which case the guideline would be calculated as 1/10 of the lowest LOEC, by the method in the paragraph above.

Some agencies in Europe have formulated some rules which are akin to toxicity guidelines, and could be used to derive water quality objectives in any given situation. In Sweden, a handbook of methods for "Characterization of Industrial Discharges" (CID) gives some guidance on dilutions which are thought to create a degree of safety (SNV 1991, discussed further in section H.3). Toxicity of an effluent is first judged by tests of acute lethality.

(a) If receiving-water concentrations will be >0.1 LC50 (dilution less than ten-fold below the LC50), the discharge is considered acutely toxic and remedial action should be taken.
(b) If receiving-water will be >0.01 LC50 (dilution less than 100-fold below the LC50), the discharge is "capable of causing damage", and sublethal tests should be done.

A similar approach is suggested by OECD (1987) for regulating effluents.

(a) If the dilution would be < 100-fold, the effluent deserves sublethal testing.
(b) If the dilution would be > 100-fold, undiluted effluent should be tested with acute tests (<7 d). If the effluent did not then cause 50% mortality, it would have a weak priority for further testing, i.e. it would not be considered very dangerous in the receiving water.
(c) Presumably, if dilution would be > 100-fold and the effluent was lethal, it would be regarded as dangerous in the environment, i.e. similar to the Swedish item (b) above.
(d) If dilution would be > 1,000-fold and 24-hour tests showed the effluent was not lethal, it should be considered of low priority for additional testing.

Neither of the European documents states or implies that dilution below a certain fraction of the LC50 means that conditions are "safe", and the Swedish document says clearly that less than 1/100 of the LC50 is not proven "safe" for aquatic organisms. Nevertheless, **there is a general implication that non-lethal effluents diluted 100-fold, are not of major concern.**

The European aquatic safety factors would represent dilution more than 100-fold below the **lethal concentration.** Since the NOEC would be expected to be at least ten-fold lower than the LC50 (section I.4.3), the aquatic safety factor would represent 10-fold dilution, or less, below the NOEC.

The European handbooks are, essentially, **using the bio-tests as prototype water quality objectives.** For example in the Swedish handbook, if the concentration in the receiving water is > 1/100 of the LC50, then the waste is considered potentially harmful. It is only a small step further to define a fraction of the LC50 or NOEC that is thought to be without harmful effect.

A Dutch recommendation of a safety factor of 10 below the NOEC for short sublethal tests is described in section J.3 (van Leeuwen et al. 1992).

These four examples of a safety factor and approximate dilution factors in aquatic toxicology have a good degree of unanimity. All of them indicate **guideline values that would be about one-tenth of the sublethal Threshold Effect Concentration**, or a somewhat lower concentration than that.

I.4.2 Recommended safety factor and manner of using it

It is recommended that a safety factor should be selected that would be suitable for use with concentrations of effluents in the receiving water, in similar fashion to the application of safety factors to the lowest LOEL for a given chemical. The choice should involve somewhat narrower and more directed consideration than can be done here, of evidence on valid safety factors. A suitable factor would probably be in the general range of 0.1.

The factor would be applied in the same way as is done at present in guidelines developed by CCME (1991). For a given effluent, sublethal toxicity tests would be done, preferably with two or more types of organisms. The safety factor might be applied to the TEC, calculated as the geometric mean of NOEC and LOEC, or another appropriate endpoint might be used.

By specifying the sublethal tests to be used and the value of the safety factor, a water quality guideline would have been defined.

I.4.3 Methods of predicting no-effect levels from lethal tests

It is possible to extrapolate data from a lethal test to approximate a very low degree of lethal action, say the LC0.01 or even the LC0.001, i.e. a concentration estimated to cause mortality of only 1% or 0.1% of the test organisms. By extrapolating again for various exposure-times (1 d, 2 d, 3 d, 4 d) it might be possible to estimate an *incipient* or *infinite* LC0.01, which would kill only 1% of organisms after an infinitely long exposure. Obviously there are strong possibilities of error in such extrapolations. The approach has been considered by other authors, since concentrations estimated to cause no long-term mortality are reasonably well correlated with measurements of no sublethal effect (Mayer et al. 1986, reviewed in Giesy and Graney 1989).

This approach is not recommended because of the uncertainties involved in two extrapolations. The new short-term sublethal tests are more desirable, since they are almost as fast as lethal tests and give direct estimates of no-effect concentrations.

A similar topic might be given acknowledgment and brief mention, although it is of secondary importance for the issues considered here. There has been a fair amount of effort expended in reviewing the ratio between concentrations causing acute lethal effects, and the concentrations at the threshold of sublethal effects.

In general, the sublethal NOEC is 1/10 of the LC50 or lower. For pulp mill effluents, Bonsor et al. (1988) summarized toxicity work by estimating that the sublethal/lethal (≈ chronic/acute) ratio was generally ≥ 1/10 (i.e. that was a conservative or "safe" average value). A few findings showed a greater spread between lethal and sublethal concentrations, and a chronic/acute ratio of 1/20 would include almost all ratios and would be a very conservative value. Hodson et al. (1991a) calculated an average ratio of 1/10 between the thresholds of sublethal effect and median lethal levels, for phenol and a variety of chlorinated organics. For narcotic organic industrial chemicals, the mean sublethal/lethal ratio for early life-stages of fish was 0.067 (≈ 1/15, van Leeuwen et al. 1990).

I.5 Variability

***Summary.** The coefficient of variation for modern sublethal aquatic tests is often in the range 20% to 40%, which is as satisfactory as the variation found for chemical tests.*

Variation of results from good biological testing is no greater than, or is better than, the variation found in chemical measurements. Precision of the 7-day test with larval fathead minnows might be taken as an example. A large and intensive test of variation was done by ten U.S. laboratories (API 1988). There was good agreement, with inter-laboratory coefficients of variation of 13% for survival of larvae and 52% for final weight. Another comparison of results from nine laboratories in the San Francisco area (Anderson and Norberg-King 1991) reported a coefficient of variation of 31% (22% when calculated properly on a logarithmic basis).

That precision in the sublethal fathead minnow tests is somewhat better than in chemical analyses of priority pollutants, for which a comparable average inter-laboratory coefficient of variation was ≥60% (Rue et al. 1988).

The U.S. EPA (1991) reviews coefficients of variation for inter-laboratory comparisons of other sublethal tests as follows.

Killifish 7-day early life-stage	44%
Ceriodaphnia dubia 7-day reproduction	21%, 29%, 41%

These coefficients are in the same general range as outlined above for the fathead minnow, and should be considered satisfactory.

I.6 Validation

Summary. Modern sublethal toxicity tests of effluents correlate well with conditions found by survey of the biota in the receiving water.

The 7-day larval test with fathead minnows may be taken as a standard sublethal laboratory test that has shown excellent correlation with ecological evaluations of polluted waters. In a Kentucky river, the degree of mortality of larval fathead minnows had correlation coefficients of 0.92 to 0.96 with the number of fish species resident in sections of the river, and with the number of invertebrate species and their diversity (Birge et al. 1989).

There has been a deliberate attempt in the U.S.A. in recent years to test whether the short sublethal tests correlate well with the observed conditions in receiving waters. The program of testing and survey has been an extensive one, largely done by U.S. EPA or sponsored by them. The results show reasonably good agreement (Fig. I.1).

The agreement in the studies of U.S. EPA was not perfect, but allowance should be made for the direct method of prediction, without the safety factor that is customary in most single-chemical water quality guidelines. For the four sets of evaluations (which have some overlap of data), the largest sectors of the pie diagrams are for agreement of a predicted and an observed impact. There was 65% to 86% agreement between a predicted effect (toxicity test) and observed effect (field survey). The white sections of the pies also show agreement, of an effect neither predicted nor observed (4% to 23%). Disagreements were shown in the other small sectors (black and lined). Impacts were predicted but not observed in 7%, 8%, 8%, and 9% of the cases., i.e. the prediction apparently allowed some margin of safety. No impact was predicted, but one was observed, in 2%, 5%, 6% and 11% of the sets of correlations, i.e. the toxicity prediction apparently did not allow a margin of safety.

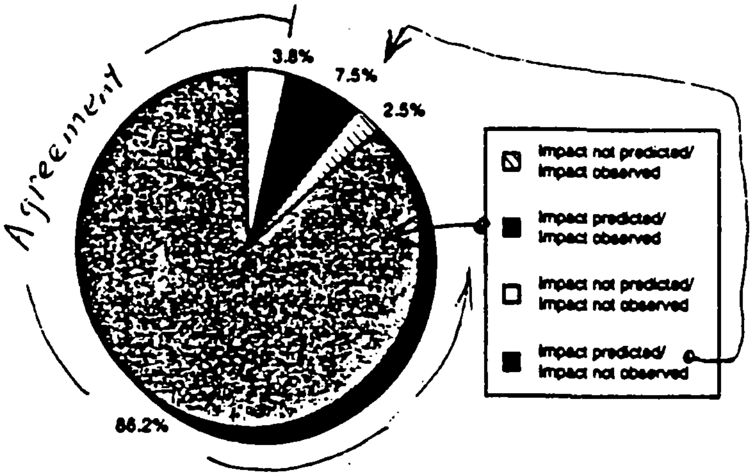

Figure 1-2. Comparison of Ambient Toxicity and Instream Impact—EPA Study [23, 31-38]

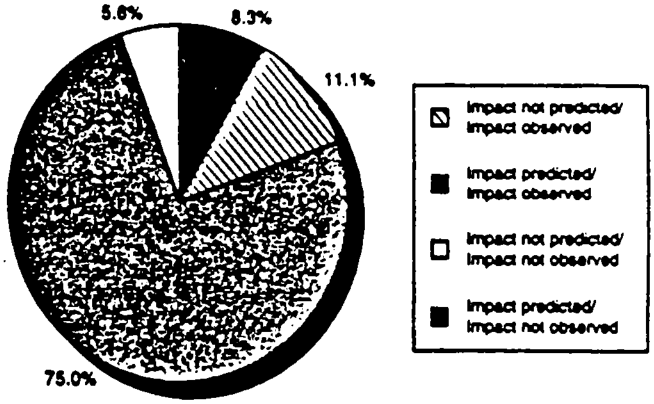

Figure 1-4. Comparison of Ambient Toxicity and Instream Impact—Trinity River [29]

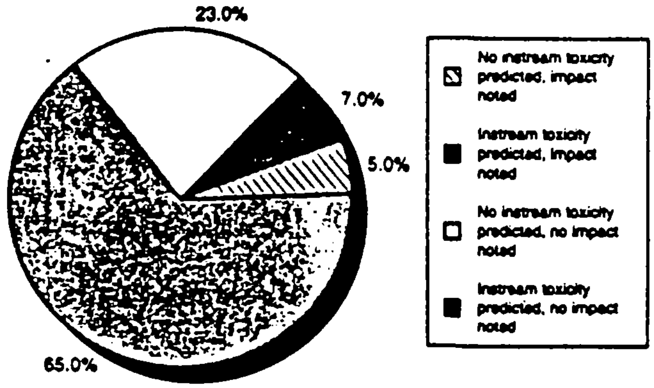

Figure 1-3. Comparison of Effluent Toxicity of Receiving Water Impact Using *Ceriodaphnia* Chronic Toxicity Tests and Freshwater Receiving Stream Benthic Invertabrates at 43 Point Source Discharging Sites in North Carolina [28]

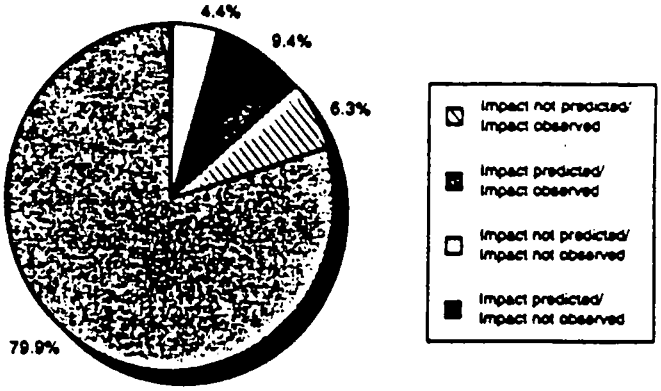

Figure 1-5. Comparison of Ambient Toxicity and Instream Impact—EPA Study, Trinity River Study, and Kentucky Study [26]

Fig. I.1. Comparison of predictions from sublethal testing of effluents, with the effects on biota of the receiving waterbodies as determined by surveys. From U.S. EPA (1991).

I.7 Expert advice

Summary. Most interviewed people were comfortable with the concept of using sublethal testing as a water quality guideline. It appears that biochemical perturbation in fish, caused by pulp mill effluent, occurs at concentrations below the NOEC for the usual sublethal tests with daphnids and fish larvae. The biochemical tests are not suitable for use in guidelines, at present.

Although some people were doubtful of the unfamiliar concept of using biological tests in water quality guidelines, most interviewees agreed with the idea and some gave strong support. One person said that biological testing makes sense because it is difficult to know what is being discharged today, and in fact we often do not know what chemicals are in the effluent.

Environment Canada has recognized this avenue of approach for setting guidelines and is pursuing the concept of toxicity tests at contaminated sites.

The standard sublethal tests may not be sensitive enough for BPME. Drs. John Carey, K. Munkittrick, Mark Servos, and their associates found that tests with *Ceriodaphnia* and fathead early life-stages simply did not predict the biochemical effects on fish that they found in the 10-mill survey of Ontario mills in 1991. The *Ceriodaphnia* tests were done on receiving water so did not achieve maximum sensitivity, but there was no effect with fathead minnows tested with 100% effluent at Espanola, Ontario. A safety factor applied to the sublethal threshold (section I.4) might be satisfactory. A series of papers by this group are forthcoming and should be sought by people who work in this field (e.g. Munkittrick et al. in press).

Carey's group (T.G. Williams) is developing a 6-day test for MFO induction in the lab, by exposing fish to effluent in water. The group adopted the hypotheses that (a) MFO induction is accompanied by changes in reproductive steroids, and (b) steroid effects in fish are the most sensitive of any effects. The assumption is that if the MFO and steroid changes are eliminated in a given location, there should be no other effects on fish.

Whether an MFO test should be used as a guideline raised different opinions. One government scientist (Dr. Lyle Lockhart, Fisheries and Oceans, Winnipeg) agreed that the EROD test was a sound and reproducible sub-cellular response, and a good indicator of exposure. He saw interpretation as a problem, however, and considered that EROD work is 4 or 5 years, maybe 10, away from understanding the meaning for the whole animals. He wondered if anyone would venture to interpret the significance of a 10-fold induction of EROD. A university researcher (Dr. G.J. Van Der Kraak) pointed out that MFO induction did not necessarily correspond to decrease in reproductive steroids and the latter govern the significant whole-body effects. Unfortunately there is a rather complex natural seasonal variation of steroids over the seasons, which must be considered in any evaluation of pollution effects.

APPENDIX J
QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS

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Summary. QSAR predicts toxicity of compounds from chemico-physical structure. Four categories are usually defined in aquatic toxicology, with the most used being the first two: (1) narcosis I type, such as 1-octanol and most of the chlorinated phenolics of interest in pulp mill waste, which cause general membrane perturbation; (2) narcosis II, such as phenol, acting by depolarization of membranes; (3) uncouplers of oxidative phosphorylation such as dinitrophenol; (4) and inhibitors of electron transport, such as hydrogen cyanide. QSARs are useful for predicting degree of toxicity, and also bioaccumulation, degradability, and joint action of similar substances in a mixture.

QSAR stands for the words used as the heading for this section, and deals with relationships between (a) the physical and chemical characteristics of a chemical, and (b) its activity in the environment, particularly toxicity. QSARs "are used increasingly to screen and predict the toxicity and the fate of chemicals released into the environment" (Nirmalakhandan and Speece 1988).

This is currently an extremely active field of research, with a continuing stream of important papers. Powerful techniques such as multivariate analysis are being applied to develop strategies for ranking chemicals (e.g. Eriksson et al. 1990).

Potential usefulness. In the past, use of the techniques of QSAR was largely limited to predicting which chemicals were the most dangerous, deserving special attention. That is still a useful role in development of water quality guidelines. Once chemicals in an effluent are identified, QSAR techniques can point to the relative degree of environmental danger that is to be expected. Use of QSAR with pulp and paper effluents could play an important role; as more of the unknown constituent chemicals are identified, QSAR could help indicate which were of greatest potential concern, and which deserved additional toxicological study. The advantage lies in a rapid and relatively inexpensive technique which uses existing models. The disadvantage of QSAR is in its predictive nature, subject to error in real-world situations.

J.1 Categories of QSAR developed to date

Four relationships are now commonly considered appropriate and are being used in evaluations of mixtures in aquatic toxicology (Broderius 1991). They show appreciable differences in their QSARs based on log K_{ow} (Fig. J.1).

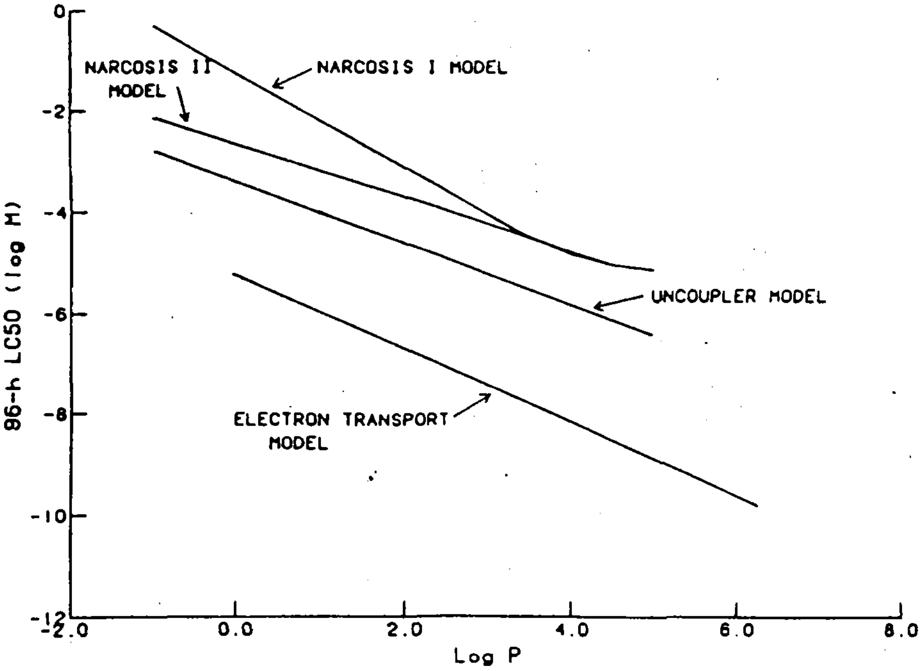

Fig. J.1. Different QSARs of acute lethality to fathead minnows, for industrial organic chemicals as related to their octanol-water partition coefficients (P or K_{ow}). From Broderius (1991).

Narcosis I. Chemicals in this category cause general membrane perturbation in organisms. These chemicals are typically industrial organic chemicals which do not have a particular biochemical toxic mechanism, but rather a general, low-grade "narcosis-like" effect. This category is frequently referred to in aquatic work, because it includes many common chemicals. A typical example is 1-octanol.

Narcosis II. These chemicals act by depolarization of membranes. An example is phenol, and many halogenated and substituted phenols fall in this category. Nonacidic substituted and highly halogenated phenols with log K_{ow} of ≥3 are additive with substances in Narcosis I and Narcosis II categories. Similar substituted/halogenated phenols with log K_{ow} <3 are additive within the Narcosis II category (Fig. J.1)

Uncouplers of oxidative phosphorylation. Interference with this enzymatic action is thought to be the characteristic mode of action of chemicals such as 2,4-dinitrophenol.

Inhibitors of electron transport. This action in the mitochondria of cells is thought to be the mode of toxicity of some substances such as hydrogen cyanide (HCN) and rotenone. Substances within this category are additive in their action but less-than-additive with the uncouplers of the third category.

J.2 Potential future use

The future usefulness of QSAR expands into a diversity of related topics. Not only toxicity, but bioaccumulation, degradability, joint action with other substances, and modelling the fate of chemicals in ecosystems, are all promising enterprises. A detailed examination of these options and existing experience is given by Nirmalakhandan and Speece (1988). In the recent past and immediate future, analyses are being provided by L.S. McCarty and colleagues in a series of papers (McCarty 1986, 1991a, McCarty et al. 1985, 1991, 1992, Mackay et al. 1992). McCarty stresses that the key to an understanding and prediction of relationships is to work towards, and work with, the dose of toxicant(s) inside the organism ("residues"), since that is the operative quantity. (This is "analogous to judging inebriation of humans by blood-level of alcohol", which is accurate and advantageous, rather than by less dependable estimates based on number of drinks.) He has also demonstrated essential parallelism of relationships for acute and sublethal QSAR (McCarty 1986).

The techniques have use in a number of topics covered in this report: persistent bioaccumulative materials; mixture toxicity; categorization of chemicals by action; and the primary use of QSAR mentioned above, of predicting which toxicants are of most concern.

A demonstration of usefulness of QSAR for various phenols is given by Hodson et al. (1991b); lethal and sublethal toxicity was clearly related to the octanol-water partition coefficient for a non-polar narcotic, polar narcotics, and an uncoupler of oxidative phosphorylation. Most of those test-chemicals were chlorinated organic materials of interest for pulp mill studies. The chlorinated phenols so important in BPME fall into this category of general, "narcosis-type" toxicity. This was demonstrated in a study of 19 chlorophenols which concluded that the effects of chlorophenols (and chlorobenzenes) "are primarily related to the chemicals' octanol/water partition coefficients" (Kaiser et al. 1984). A similar QSAR for many relevant chlorophenols was also determined by Devillers and Chambon (1986).

J.3 Setting water quality guidelines by QSAR?

Summary. For narcosis I chemicals, researchers from The Netherlands concluded that knowledge of molecular weight and octanol-water partition coefficient allows prediction of the toxic concentrations in water, sediment, and tissues. The internal toxic concentration (ITC) of this category of substances, for fish, which was judged to protect 95% of the species in a community, was estimated as 2 x 10^{-5} moles/kg. The Dutch researchers generalized that a safety factor of 10, below the lowest whole-organism NOEC in the laboratory, estimated the ITC.

The joint toxic action of non-specific toxicants is essentially additive, so the ITC given above applies to the total body burden from a mixture. Dutch practice is to use an arbitrary safety factor of 100 to allow for all substances present and reach a "negligible risk level". QSARs could be used in at least predictive and supporting roles, to develop guidelines for chemicals in BPME.

Van Leeuwen et al. (1992) demonstrated the rapid advances being made in the field of QSAR. The paper was based on toxicity data for 102 organic chemicals with 19 species of aquatic microbes, algae, invertebrates and vertebrates. From molecular weight and K_{ow}, predictions were made of toxicity and other ecotoxicological data. The chemicals included a series of alcohols, chlorinated benzenes and toluenes, diverse ketones, ethers, and chlorinated alkanes.

From an amalgamation of empirical observations, theoretical calculations and generalizations of current information, the authors predicted relationships that are of great practical interest. They published a table which can be entered by using K_{ow} for a non-polar organic substance acting by narcosis, and which then provides estimates of concentrations to protect 95% of the species in a community from sublethal effects (the **HC5**, or Hazardous Concentration for 5% of the species). Specifically, estimates are provided for several compartments of a community:

- HC5 for the fraction of the chemical dissolved in water;
- HC5 for the total waterborne fraction (i.e. in water + suspended solids, etc.);
- HC5 for the fraction in sediment; and
- HC5 for residues of the substance in living organisms.

The *internal toxicant concentration (ITC)* for threshold effects was assumed to be a constant, for these relatively unreactive, non-polar, narcotic chemicals, a concept which agrees with work of earlier investigators. Van Leeuwen et al. (1992) estimated that for protection of 95% of species, the ITC for fish is 2 x 10^{-5} moles/kg. They concluded that this ITC "may be seen as a water-quality standard".

They gave a further generalization that a **safety factor of 10**, below the lowest NOEC derived from tests of reproduction of *Daphnia* and early life-stages of fish, would be expected to estimate the HC5. They pursued the concept further by considering the **joint toxic action** of these substances of similar generalized effect, shown to be essentially additive by previous Dutch (and other) work. Obviously the toxicity of mixed pollutants would depend on how many were present and at what concentrations, but the authors suggested that as a practical resolution, it has been the practice in The Netherlands to apply an arbitrary safety factor of 100 to the single-chemical HC5, to allow for all substances present and reach a "negligible risk level".

J.3.1 Inferences and suggestions

The preceding discussion in section J.3 was based on only one paper (van Leeuwen et al. 1992), but it gives an indication of the relatively advanced use of QSAR for integrating and modelling organic pollutants in aquatic systems. It seems clear that the generalizations being developed will receive wider use in the near future. It appears that the techniques, despite their approximations, will receive general acceptance. QSAR modelling of narcosis-causing organics could be used to supplement the actual toxicity data for individual substances in BPME when developing water quality guidelines.

J.4 Advice from interviewees

Summary. Most people interviewed did not volunteer QSAR as part of developing water quality guidelines. A few interviewees urged caution in using QSAR. Using body burdens would avoid many problems of predicting from the laboratory to real systems. There is a practical problem of dealing with many compounds in an effluent, as in BPME. Other interviewees were using QSAR to predict dangerous substances, or agreed that mixture toxicity could be estimated by using QSARs.

Most of the people interviewed did not think to include QSAR as one of the tools for developing water quality guidelines. Thirteen interviewees did not volunteer anything on the subject. Among the others, sometimes prompted by questions, three people suggested caution.

> Dr. Hodson of Fisheries and Oceans urged suitable scientific (cautious) approaches, despite the excellent and convincing predictive relationships shown by his work. "Do QSARs from one species apply to other species? Dr. L.S. McCarty showed that QSARs in fish do not tie in so well with the real world, because of all the other factors that come into play. Volatility, decomposition, etc., are all going on at the same time as the QSAR with toxicity. By looking at the **dose in the animal** you get around the problem".
>
> Dr. Rogers of Fisheries and Oceans. He "really questions this approach" ... does not "really think it will work out." Once you start talking of measuring everything that is present, "you are talking [about] an army of researchers to deal with one effluent".

Dr. McKague of Pulp and Paper Centre. "This is rather speculative. Not really suitable for water quality guidelines."

Three others gave positive comments about using QSAR.

Henderson of B.C. MOE was actually using the approach. She was "trying to pick 'indicator compounds' ... and **using QSAR to assign priorities**, focusing on organochlorines".

Dr. Halfon of National Water Research Inst. has worked on QSAR. He wrote a paper ranking chemicals for hazard using a multivariate approach (Halfon and Reggiani 1986). Physico-chemical factors were assigned, and a series of chemicals emerged in a diagram, with the most dangerous ones near the top. The paper included groups of chlorinated phenols and benzenes.

Richard Lloyd, a long-time toxicity worker in the U.K., supported use of QSAR to predict toxicity of narcotic-type chemicals; perhaps toxicants with similar mechanisms will add together in toxicity like the Type 1 narcotics that fit K_{ow} (Lloyd 1991a, p.227).

J.5 Additional background description of QSAR

For numerous organic chemicals which have a very generalized toxic effect ("narcosis"), there is a strong relationship between physico-chemical properties and toxicity. The octanol-water partition coefficient is correlated with degree of toxic effect. Fig. J.2 shows an example for aliphatic alcohols, with log LC50 linearly related to log P [= log K_{ow}] over most of the range. Ketones, ethers, alkyl halides, and substituted benzenes fit the same relationship, whether test fish are fathead minnows or guppies (Veith et al. 1983).

Furthermore, sublethal toxicity roughly parallels the lethal relationship (McCarty et al. 1985). Such correlations are described as **quantitative structure-activity relationships** or **QSAR**, and they give hope of sorting out dangerous organic substances from the flood of new chemicals which come into use every year.

Substances with specific toxic mechanisms (insecticides, pentachlorophenol, etc.) are much more toxic than indicated by the line in Fig. J.2. More varied and esoteric physico-chemical characteristics must be considered in such cases, and predictive relationships are not well established. Some progress has been made in grouping the metals (Kaiser 1980).

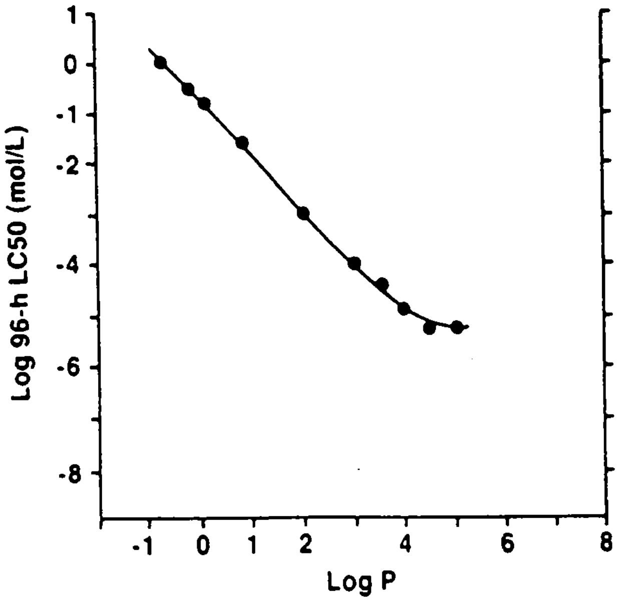

Fig. J.2. Relation between the 96-h LC50s for fathead minnows, of a series of aliphatic alcohols, and the octanol-water partition coefficient (P or K_{ow}). Modified from Veith et al. (1983).

J.5.1 QSAR for degree of chlorination of organic molecules

Some chlorinated organic substances are very toxic, but as yet there is no simple or well-defined relationship of chlorination to toxicity. Some changes tend to occur when compounds become chlorinated, and Sprague and Colodey (1989) summarized the general trends in the following way.

- becomes more toxic [this has been disputed, see section K.2]
- becomes more lipophilic and bioaccumulative
- becomes less biodegradable
- becomes mutagenic

As an example supporting the above generalizations, Sprague and Colodey cited work in the literature showing that the growth of five species of marine algae was inhibited by organochlorines in direct proportion to the numbers of halogen atoms in the substances tested.

The more highly chlorinated compounds may not always be more bioaccumulative, as shown by literature reviewed by Sprague and Colodey (1989). Large highly-chlorinated molecules like octachloro-dibenzo-dioxin have difficulty in crossing bilipid membranes, and hence do not accumulate as rapidly as predicted by their octanol-water partition coefficients. Other studies have found a correlation between the bioconcentration factor of a compound and its triolein-water partition coefficient. Nevertheless, some high-molecular-weight chlorinated compounds can degrade into chlorinated phenols, catechols and guaiacols, then be transformed biologically into very toxic and bioaccumulative compounds like anisoles and veratroles (Allard et al. 1988).

APPENDIX K
MODELLING AS A LINK IN DEVELOPING GUIDELINES

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Overall Summary. Modelling is yielding many advances in predictive ability for bioaccumulation, mixture toxicity and QSAR, particularly if it deals with dose of toxicant within organisms. British Columbia has a major project for arriving at regulatory control of toxicant loading from pulp mills, and modelling of toxicant behaviour is a key component. Despite all this, a direct role in development of water quality guidelines was not immediately apparent. There should be a narrower and more explicit examination of how modelling could be used to develop guidelines.

This section deals primarily with mathematical modelling as a means of relating toxicant residues in organisms, to water quality guidelines. However, modelling enters into many other topics of this report, particularly Toxicity Equivalents (Appendix F), Mixtures (Appendix G), QSAR (Appendix J), and blends directly into Bioaccumulative Substances (Appendix E).

K.1 Toxicological modelling, general and pulp-mill-related

Summary. Modelling is a powerful predictive and integrative tool, with some successes in assessing residues of organochlorines (dioxins) from pulp mills.

Only a brief outline of modelling is given here, pointing out some aspects relevant to effects of BPME. There is broad usage of modelling techniques, for example many recent studies in mammalian toxicology use "PB-PK" modelling ("physiologically-based pharmacokinetic modelling"). Reviews on pharmacokinetics in aquatic animals delve directly into uptake, residues, and QSAR.

For pulp mill effluents, modelling has included extensive prediction for chlorinated dioxins and furans in fish (Muir et al. in press). These substances are not included in the present report, but the accurate predictions with them bode well for attempts with other components of mill effluent.

K.2 Modelling the dose within organisms

Summary. Rapid future progress in dealing with complex mixtures of organic pollutants will be fostered by evaluation based on body burdens rather than concentrations in the surrounding water.

This topic ties in very closely with Appendix E which is concerned with persistent bioaccumulative substances, but internal doses of toxicant are important in broader ways.

Dr. Lynn S. McCarty has expanded on the topic that major improvements in understanding and predicting aquatic toxicological relationships, will require working with the body burdens of toxic chemicals instead of the external concentration in the water ("it is dose that counts"). His research on the topic has already been a strong force for unifying findings on acute versus chronic toxicity, bioconcentration versus toxicity, and structure versus effects (McCarty 1991a, McCarty et al. 1991).

McCarty related his approach to toxicity of bleached pulp mill effluents at a recent conference at University of Toronto ("Why do regulations for organic chemicals focus primarily on water-borne levels rather than organism levels?", McCarty 1991b). His main point was the predictive advantages of working with dose, but he gave many specific examples, such as the ease of assessing joint toxicity of similar substances, and the generalization that chlorinated hydrocarbons are not inherently more toxic than non-chlorinated ones.

Opinion in the literature, from leading aquatic toxicologists, supports McCarty's premises. One problem is lack of background data on dose-related toxicity in the aquatic field, and the other problem appears to be a reluctance of investigators to move from the traditional concept of water-related toxicity. Certainly Dr. Frank Gobas, associated with the effluent program in B.C. (see section K.4), was clear in his support for dose-related approaches. When interviewed, he responded that there was great promise for a profitable predictive/modelling approach. He saw the main limitation as lack of data, making it difficult to determine whether a substance had the 'minimal' or 'standard' toxicity that prevails in a certain category of chemicals (e.g. 1 to 6 μmolar/kg), so that it can be modelled easily, or whether the chemical has a more specific toxic effect and will cause effects at a lower concentration.

K.3 Potential difficulties with models

Models tend to become outdated rapidly, and accordingly it might be difficult to incorporate particular mathematical models into water quality. Specifying a general approach is better, but for guidelines this has the disadvantage of being indeterminate. A guideline incorporating modelling would have the problems that would come in the absence of a fixed set of values.

One of the interviewees raised points that were more specific to BPME (Dr. J. Carey, Fisheries and Oceans, Burlington, Ont.). He pointed out that much of the present-day modelling in aquatic toxicology tends to be based on a DDT/PCB pattern. Very little of the toxic material in pulp mill wastes behaves in that way. Many substances have a negative charge, and exposure depends very much on the character of the water.

Another point was that the disappearance rate of MFO-inducers in BPME is very site-specific, and is apparently related to the type of community present, with bottom substrate and biofilm being important. There is a problem, accordingly, in generalizing for more than 200 mills in Canada. That is a major issue when considering uses of modelling for **national** water quality guidelines.

Another caution expressed was that allowance must be made for metabolism of toxic components of BPME. Since there is transformation within living organisms, substances in the tissues could differ from those present in the effluent itself.

Potential usefulness. At this time it would seem that a major role for modelling is predicting the relative harmfulness of substances in BPME, how they are likely to act, and where they should be found. This in itself is a major benefit, which should allow investigators to focus on the major problems with a minimum of wasted effort. Modelling can help confirm patterns that may be in an early state of development with regard to empirical data.

K.4 The B.C. Ministry of Environment project

Summary. The British Columbia Ministry of Environment is actively engaged in developing an approach which uses modelling as a bridge between residues in fish and the discharge loading. At present the B.C. project is not developing environmental quality guidelines but is (a) a regulatory exercise, (b) concerned with human health rather than the ecosystem, and (c) site-specific.

A current project of B.C. MOE involves Dr. John Ward, Nadene Henderson, and Dr. Frank Gobas (Simon Fraser University). This programme is concerned with organochlorines, and is scheduled to run for two years. It responded to a provincial government request for research to support the establishment of regulatory controls for organochlorine compounds from pulp mills.

Major efforts so far are apparently taking advantage of approaches designed to protect humans from organochlorines in fish tissues. There are three main steps in this work, according to the early pages of draft no. 12 of a conceptual plan for the work (B.C. MOE 1991).

(1) Available chemical and toxicological information on pulp mill organochlorines is being compiled.
(2) Acceptable limits in fish tissue will be adopted or ascertained.
(3) Modelling will be used to determine the **loading** that is permissable from effluents, to stay within the tissue limits.

It seems clear from the first part of the B.C. plan [20], that the major early objective is to **develop a method of regulating effluents** that is scientifically valid, as well as being effective, but developing guidelines for the receiving water is not a primary objective.

K.4.1 Some details of the approach

The project deals with persistent bioaccumulative materials, and starts with guideline levels in fish for the protection of human health. From there it works through modelling of water carriage, back to the allowable **loading** from effluent. The group considers the approach to be quite feasible, and in particular, Dr. Gobas says that the modelling part in the middle is workable.

Current activities (1992.January) involved compiling a database of chemical and toxicological information on components of BPME, focusing on organochlorines. "Indicator compounds" will be picked, e.g. one might regulate for guaiacols, and assume that would satisfactorily control the other things. QSAR are being used to assign priorities, for substances to be included in the library of information.

For each substance in the list, ten items of information are searched for and compiled: (1) concentration in effluent; (2) mammalian toxicity; (3) fish toxicity; (4) invertebrate toxicity; (5) algal toxicity; (6) K_{ow}; (7) BCF; (8) formula to convert BCF to K_{ow}; (9) half-life in the environment (for some substances); and (10) items missed. From that information, substances are assigned to one of three categories: (a) known/characterized; (b) incompletely characterized; and (c) "mystery substances" (which may have to be neglected).

Certain limitations were brought forward by members of the team in B.C.

- The project is starting with human health guidelines. Doing a parallel exercise for health

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[20] Only the first part of the plan is outlined here, the part that is under way. After that, the plan expands greatly into ecosystem protection, but it seems unlikely that it will be possible to undertake the ambitious later stages. The words of the plan have an extremely wide scope. For example, one paragraph outlines a research plan which would include "on-site and in-lab" work on "effluents, sediments, and water with effect on aquatic species ... representative of all ecosystem trophic levels", including effects of "different OC components [organochlorine components] on these species", with effects on "enzymes, hormone levels, and population levels of fish" and "other selected species". It is not clear whether the required ecotoxicological expertise will be available to design and support the plan drawn up by the authors.

(protection) of aquatic organisms would be more difficult and lies in the future. Dr. Gobas was confident the system would serve for protecting aquatic organisms but we need considerably more information relating levels in fish to effects (section K.2).

- The modelling has to be very site-specific. It will involve currents, dilutions, and many more subtle things. For this reason it is good for regulation of individual mills, which is what the group is working towards.
- This may not provide all-purpose water quality guidelines, either numerical or narrative.

K.4.2 Guidelines for concentrations in the water?

Despite the last item in the preceding section, concentrations in the water are apparently to be considered. Interviews indicated that from a starting-point of water concentrations, the concentrations in fish tissue will be predicted. They are depending on models to associate environmental concentrations with "safe" limits of residues in fish for human health. [There could have been a misunderstanding about starting with the water concentration, during transcription from telephone conversations. Other information (above) indicates a start from residues in tissue, then modelling back through the water.]

The early part of the draft plan has an objective of determining limits for loading from effluent. Dr. Gobas feels that the approach is less suitable for developing water quality guidelines (see preceding section). On page 7 of the 8-page text of the plan, however, it appears that there is also an intention to recommend "criteria for OC levels in sediments, water, and biota". Interviews indicated that the group is aiming at receiving-water guidelines for (a) whole effluent, (b) AOX fractions, and (c) 10 to 20 specific substances as indicators.

K.5 Expert advice

Summary. A majority of people supported the concept of using mathematical modelling to bridge parts of a process setting guidelines, and thought the modelling would gain general acceptance.

There was general interest and some degree of approval, for linking bioaccumulative toxicants ("residues") in aquatic organisms with loading by an effluent, by means of mathematical modelling. Among those interviewed, most felt that the approach would find acceptance for developing guidelines and also for regulatory use.

Dr. Efraim Halfon at the Canada Centre for Inland Waters has built a program which emulates Lake Ontario. One can manipulate inputs of pollutant, type of treatment, and other factors, then visually display the predicted concentrations in a plume, or beyond a river mouth. The model could be adapted to other bodies of water, given time, but is quite specific to the design body.

It is better for big lakes. This Environment Canada model has 7 compartments (water, sediment, "plankton", detrital plants, benthic invertebrates, small and large fish). Some real characteristics of the food web must be used to get a meaningful prediction. Dr. Halfon has data in the file for about 6 chemicals, including chlorinated benzenes and pesticides. Although this model may not be the one which could assist in developing national water quality guidelines for BPME, it could certainly be used to evaluate their usefulness more locally, i.e. in Lake Ontario.