EVALUATION OF ECOLOGICAL RISKS ASSOCIATED WITH THE USE OF CHLORINE DIOXIDE FOR THE BLEACHING OF PULP

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EXECUTIVE SUMMARY

This review and subsequent report were conducted at the request of the Alliance for Environmental Technology by a Scientific Review Panel specifically convened for the purpose. The task put to the Panel was to review and summarize the known or plausible impacts and, where possible, quantify the risks to aquatic organisms and ecosystems associated with the discharge of treated effluent from pulp and paper mills using 100% chlorine dioxide substitution (ECF). Also included were several other issues related to the environmental impacts of pulp production and bleaching. This was done with special reference new information that had become available since the last review which was conducted on data available up to 1993. In this process, the panel used a weight of evidence approach and followed an approach that was similar to the risk assessment frameworks developed by the U.S. Environmental Protection Agency and Environment Canada.

The risk assessment completed in 1993 concluded that "mills bleaching with high chlorine dioxide substitution (100%), employing secondary treatment and with receiving water dilutions typical of most mills in North America, present an insignificant risk to the environment from organochlorine compounds." The authors of the 1993 risk assessment identified several gaps in our knowledge of the fate and effects of pulp mill effluent from mills: For example; (i) the substances responsible for liver mixed function oxidase (MFO) enzyme induction and reductions in serum concentrations of reproductive steroid hormones in fish exposed to biologically treated pulp mill effluents from mills with and without any form of bleaching had not been identified; (ii) the sublethal responses in fish at the population level required further investigation, and (iii) more identification and quantification were required of organochlorine compounds in effluent from mills using high chlorine dioxide substitution.

In this review, the panel identified two major questions that have formed the basis for report. These are:

> What are the risks attributable to the additive or non additive components associated with all aspects of mill operations that result from the final release to the aquatic environment of pulp mill effluents using 100% ClO_2 substitution?
>
> and, what are the specific risks, if any, contributed by the use of ClO_2 as opposed to other factors in pulp and paper production?

With regard to the second, and narrower of these two questions, and in confirmation of the conclusions of the 1993 review and assessment, the conclusions of the Panel with respect to chlorinated substances are straightforward. The clear weight of the evidence is that bleaching with 100% ClO_2 substitution (ECF bleaching) produces chlorinated substances, such as mono- and di-substituted chlorophenols. These are similar in composition and structure to naturally occurring chlorinated substances, and, as opposed to compounds with three, four, or more chlorine atoms in the molecule, are invariably less persistent and less bioaccumulative. The environmental effects, persistence, and modes of degradation in the environment of these chlorinated substances are well understood. Exposure concentrations of chlorinated substances detected in mill effluent continue to be generally low and do not suggest that acute or chronic effects will result from their presence. This risk assessment for chlorinated substances produced as a direct result of bleaching using ClO_2 reconfirms the earlier conclusion that the chlorinated substances produced as a direct result of bleaching using ClO_2, and subjected to secondary biological treatment, present a negligible (insignificant) environmental risk to aquatic ecosystems.

The previous review noted declines in the concentrations of 2,3,7,8-substituted dioxins and furans in pulp mill effluent from mills using ECF bleaching as well as in organisms in the receiving environment. The continuation of this trend clearly reflects marked decreases in environmental inputs resulting from improvements in technology, including the use of ECF bleaching of pulp. Most North American mills have already achieved virtual elimination of 2,3,7,8- TCDD and similar reductions in 2,3,7,8-TCDF emissions. Corresponding declines in tissue concentrations of these substances in marine fish and shellfish populations have also been documented. Regional differences in the rates of reduction are likely due to differences in the dynamics of existing sediment contamination and are not a reflection of continuing input.

The only other substance which potentially could be introduced into the effluent stream specifically due to use of ClO_2 is chlorate; however, this is readily removed during the treatment of final effluent and therefore is not judged to be an environmental concern at any North American pulp mill.

The potential environmental responses to chlorine-containing compounds produced or released during the manufacture of pulp bleached by technologies using 100% chlorine dioxide substitution must be judged in relation to the production of natural background levels of chlorinated organic compounds. This production varies, depending on the geographical location, but it should be recognized that organisms have evolved in environments with background concentrations of natural chlorinated organic compounds. Many compounds, identical, or similar to those produced as a direct result of bleaching using ClO_2, are also formed as a result of natural processes. Therefore, it is not surprising that there is evidence that organisms possess mechanisms for the effective breakdown of these chlorinated substances.

As a consequence of this, a fundamental change in thinking is required – that is – to dispel the notion that only chlorinated compounds are of primary importance. Nonchlorinated compounds should be regarded as being at least equally important.

The 1993 review pointed out that a more complete characterization of all the substances produced in ClO_2 bleaching and their releases to the environment was needed. Since this time, there has been considerable progress in the area. We also have a more extensive knowledge-base of the physical, chemical and biological behavior of the classes of substances produced by this bleaching process. There are, however, many unidentified substances present in pulp mill effluent, both from ECF and TCF bleaching, such as, in the latter case, those using oxygen based chemical bleaching by hydrogen peroxide and ozone. Identification and chemical characterization of these substances should be an ongoing process.

Overall, it is not possible at present to confidently conduct a quantitative risk assessment of the larger suite of non-chlorinated substances released in pulp mill final effluent. Information on the identity and ranges of concentrations in effluent of many of the organic constituents, especially retene and abietic acid, various resin acids, and plant sterols, or knowledge of their toxicological significance is either lacking or incomplete.

The previous review noted that significant changes in the treatment of effluents from pulp mills had occurred. The presently available studies on degradation and removal processes for constituents of pulp mill effluent reemphasize the importance of effective secondary treatment in the reduction or elimination of acute toxicity in final effluent before it is released to the receiving environment. Subsequent to past improvements in kraft mill effluent quality realized through ClO_2 substitution, future improvements might be achieved through improvements in secondary treatment, since; i) recent studies suggest that many biologically active compounds in final effluent are nonchlorinated compounds contributed by pulp mill processes other than bleaching, and; ii) most of the known deleterious substances in pulp mill effluent are amenable to degradation and/or removal through adsorption to particulates. Increased recycle and spill recovery within pulp mills may also be a viable means for the continued improvement of effluent quality.

The larger question regarding risk due to the discharge of pulp mill final effluent, i.e., the known or plausible impacts associated with all aspects of mill operations including ClO_2 bleaching, pulping and all other processes, is more complicated. It is clear that recent research on the biological effects of pulp mill effluents has undergone a switch in emphasis with regard to contaminants of concern — to substances more directly associated with the pulping as opposed to bleaching process. Studies completed since 1993 suggest that some of the compounds found in treated pulp mill effluent are similar to resin acids, plant sterols, or naturally-occurring PAHs (e.g., retene) produced or released during the decomposition of plant materials. The ecological risks associated with release of such compounds in final effluent remain unresolved. While it is recognized that many of these substances occur naturally in the environment, a major difference between those released naturally and many of the constituents of effluent released from some pulp mills may be in the rates of input and concentrations produced, at least close to the point of effluent discharge.

There have been a large number of field and laboratory studies since 1993 on contaminant loadings and biological effects from pulp mills, but, the study designs have limited the extent to which cause and effect relationships can be discerned. The results are nonetheless instructive in understanding the potential for environmental impacts from pulp mill final effluent discharges, such as, for example, the lack of correlation between bleaching process and response in fish. Two of the most consistent responses of some fish species exposed to pulp mill effluents continue to be changes in MFO activity (induction and inhibition), and alterations in serum concentration of reproductive steroid hormones. These appear to be separate and unrelated responses of fish to pulp mill effluents.

While the hormone response may be the mechanism by which pulp mill effluents affect reproductive processes of some fish at some mills, the specific details are not completely understood and the population-level consequences are unclear. The toxicological significance of increased MFO activity is related to prolonged elevation of MFO activity. The increase in enzyme activity may result in greater degradation of physiologically important endogenous substances, such as hormones. In addition, prolonged gene activation may result in the diversion of energy and substrates away from normal growth and developmental processes.

Change in MFO activity in fish is a common response to exposure to many pulp mill effluents, and not a characteristic unique to bleaching. This response is most likely caused by more than one substance. The MFO-inducing substances in biologically-treated effluent appear to be natural extractives of wood released by pulping, their metabolic by-products, and/or their chlorinated derivatives. These inducing substances appear to be nonpersistent and easily metabolized and excreted by fish. The number and relative amount of these substances produced as a result of ClO_2 (ECF) or TCF bleaching is unknown.

Apart from complete ClO_2 substitution and secondary treatment, none of the studies reviewed established a clear link between toxicity reduction and installation of specific technologies. Secondary treatment alleviated acute toxicity to fish and invertebrates in laboratory tests, but adverse responses in some fish in receiving waters were still evident, although reduced. Improvements were associated with multiple changes to many aspects of a mill's operation, and could not be ascribed to any single improvement in technology. Field studies have been useful in assessing the aggregate response of organisms to effluent exposure. However, the nature of field studies, in which different observations can only be correlated, and in which numerous factors act simultaneously and independently, prevents the identification of clear cause-effect relationships. Future advances in this area may benefit from mass balance approaches that account for volumes of both liquid and solid wastes and more thorough characterization of the substances present in the effluents.

In general, responses of aquatic communities and populations to improvements in waste treatment and pulping processes have been positive, however, changes have not been sufficient to alleviate reproductive responses in some fish species in all cases. It is clear that the largest environmental gains have come from 100% ClO_2 substitution and more widespread use of effective secondary treatment. Still needed, is some attention to the nonchlorinated substances released during pulping and bleaching. These substances will probably be responsive to source control measures. Any risk management strategies employed should be evaluated on a holistic basis, for example, using risk assessment, life cycle analysis, and environmental cost/environmental benefit procedures.

The effects of long-term exposure to pulp and paper mill effluents are deserving of continued evaluation because of their introduction into receiving systems. This recommendation is made since our evaluation “tools” and “strategies” are constantly evolving and improving. However, any decisions based upon this monitoring must be made with a clear understanding of the ecological relevance of the data. Long-term and continuous exposure to effluents may cause changes in reproductive cycles, reduced or enhanced growth, changes in the age-structure of populations, or impairment of an organism's ability to cope with stress. However, all of these effects should be evaluated in the context of their larger environmental significance.

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1 INTRODUCTION

1.1 SCOPE AND OBJECTIVES

A small number of viable options are presently available for the bleaching of wood pulp (delignification and brightening) during the manufacture of pulp and paper. The use of chlorine dioxide (ClO_2) in the bleaching process has largely superceded the earlier use of Cl_2, or elemental chlorine, in the majority of North American pulp mills: It was discovered over a decade ago that the previous widespread use of Cl_2 contributed to the production and subsequent release to the environment of specific forms of potentially deleterious and highly persistent chlorinated organic contaminants, such as polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans.

The environmental risks and benefits associated with the virtual elimination of Cl_2 in pulp and paper manufacture, and substitution during bleaching with ClO_2 have been formally examined by many scientists; see for example papers by Axegård *et al*. (1993), Berry *et al*. (1991), Carey *et al*. (1993), Luthe *et al*. (1992), Munkittrick *et al*. (1996), Södergren (1989), Solomon *et al*. (1993), and Tana and Lehtinien (1996). In particular, Solomon *et al*. (1993) conducted a review and risk assessment of the chemistry and biological impacts associated with a 70% to 100% substitution of elemental chlorine with ClO_2 at the request of the Alliance of Environmental Technologies.

Major conclusions from that review are as follows:

- The reaction of lignin with ClO_2 occurs by different chemical processes than the reaction of Cl_2 with lignin. Substitution of Cl_2 by ClO_2 results in great reduction in production of highly chlorinated organic byproducts,(five to ten-fold less) and even greater reductions in dioxins and furans. This has been widely observed in effluent monitoring data.

- Much of the chlorinated organic material released in effluent from bleach kraft pulp mills, and which constitutes the major portion of "Adsorbable Organohalogens" (AOX) or "Extractable Organohalogens" (EOX), is water soluble with very limited lipophilicity, and has little if any tendency to bioaccumulate in aquatic food webs. A clear distinction is required, therefore, between different kinds of organochlorines.

- Toxicity test results for compounds found in final (treated) effluent from pulp mill employing a high degree of ClO_2 substitution do not lead to the expectation of toxicity in receiving waters typical of North American mill operations.

- The weight of evidence overwhelmingly indicated that a high rate of substitution of Cl_2 by ClO_2 will result in reductions in the quantities of organochlorines produced, degree of chlorination in individual molecules, degree of environmental persistence, potential for bioaccumulation and food web transfer, potential toxicity, and adverse ecological effects.

- Finally, the Panel concluded that, pulp mills employing 100% ClO_2 substitution, employing secondary treatment, and discharging into receiving waters with dilution factors typical of most North American mills present an insignificant risk to the environment from organochlorine compounds.

In the same review (Solomon *et al*., 1993), a number of areas of uncertainty based on the state of knowledge up to 1993 were also identified. In particular, it was concluded that -

- More knowledge was required of the variability in concentrations of organochlorines in biologically treated effluents from mills using high chlorine dioxide substitution, and of whole effluent toxicity (either as acute lethality or measured through sub-acute effects).
- The substances responsible for MFO induction and plasma steroid reduction in fish exposed to biologically treated pulp mill effluents from mills with and without any form of bleaching had not been identified.
- The risks from substances present in effluents produced from mills using "chlorine free" bleaching techniques had not been formally examined.
- More identification and quantification were required of organochlorine compounds in effluent from mills using high chlorine dioxide substitution.
- The sublethal responses in fish at the population level required further investigation.

Following completion of the 1993 review (Solomon *et al*., 1993), some interest groups have expressed the concern that replacement of Cl_2 by ClO_2 in the bleaching of pulp — while responsible for a reduction in the production rate of organochlorine byproducts — has not curtailed their production completely. Nor, according to some, can bioaccumulation of organochlorines from effluent for pulp mills using 100% ClO_2 substitution be ruled out. One of the major points of contention between the most polarized of the factions is whether 100% ClO_2 substitution has resulted in "only marginal environmental improvements", or whether such substitution is sufficient, and when coupled with effective secondary treatment of effluent represents an example of the "best available technology" for pulp and paper production.

Four years have elapsed since the 1993 review — a period in which the actual monitoring data for pulp mills employing 100% ClO_2 substitution has more than doubled. Considerable additional research has been completed on the fate and effects of pulp and paper mill effluent. This review builds on the content of the previous report (Solomon *et al*., 1993). It critically evaluates the previous conclusions, and addresses the key questions above along with other issues that have recently been identified as of potential importance. Like the previous review, this report is focused on the effects of pulp mill effluents in the aquatic ecosystem (including possible effects on wildlife via bioaccumulation through the food chain). It specifically excludes air emissions and other environmental impacts external to the mill, such as those associated with production of wood and the impacts of products exported from the mill.

1.2 REVIEW FRAMEWORK

Inherent within an assessment of the possible environmental risks associated with pulp and paper operations that utilize 100% ClO_2 substitution during bleaching are two separate questions:

> What are the risks attributable to the additive or non additive components associated with all aspects of mill operations that result from the final release to the aquatic environment of pulp mill effluents using 100% ClO_2 substitution?
>
> and, what are the specific risks, if any, contributed by the use of ClO_2 as opposed to other factors in pulp and paper production?

Both questions are directly relevant to understanding the larger environmental consequences of pulp and paper mill aqueous discharges; however, the second question is the one which is most directly relevant to risk management efforts centered around environmentally persistent organochlorines. We have attempted to address both questions here, and have endeavored to distinguish between the two issues.

It is also possible, although often more difficult, to compare the relative environmental risks of two or more operating practices. For example, implicit within an assessment of the risk of 100% ClO_2 substitution for Cl_2 is an assessment of the relative risks of bleaching using elemental chlorine versus chlorine dioxide. It is important to note that this document is not intended to provide a relative comparison between the risks associated with broadly different pulp and paper practices, such as the use of 100% ClO_2 substitution during bleaching (commonly referred to as "elemental chlorine free", or ECF bleaching) versus totally chlorine free, or TCF bleaching.

A number of regulatory jurisdictions have begun to implement environmental risk assessment processes based on set frameworks. Environment Canada is has developed a framework similar to that used in the U.S. Environmental Protection Agency (U.S.EPA, 1992) and illustrated in **Figure 1** (Environment Canada, 1996). This framework was used as the basis for our assessment.

This process involves the following:

- Characterizing of the possible stressors and their entry into the system including their sources in anthropogenic and natural processes.
- Characterizing the exposures of aquatic biota to these stressors and the fate of these stressors in the receiving environment.
- Characterizing the effects of these stressors on individual organisms at the population and ecosystem level.
- Assessing the risks of these stressors in the receiving environment and relating this to ecological theory and to management practices.
- Considering the implications of background concentrations of chlorinated substances, both natural and anthropogenic, in the environment.

The Framework for risk assessment in the Canadian Environmental Protection Act (CEPA) explicitly recognizes that organisms resident in areas where background concentrations of naturally occurring substances are elevated are likely to be more tolerant to those substances (Environment Canada, 1997). This must be taken into consideration in the risk assessment process. This is particularly important in the risk assessment of substances found in pulp mill effluent and applies to both the naturally occurring substances and chlorinated and other substances produced during bleaching and other processes. Therefore in this assessment, we have also considered the presence of naturally occurring chlorinated and other substances.

As a prerequisite to estimating the risk associated with any input to the environment, one must understand both the major compounds involved (Chapter 2), and the immediate and long-term fate of those compounds. Constituents of pulp mill effluent may be placed in one or more of a small number of categories according to their molecular and, hence, environmental properties. The categorization is, in turn, useful for devising broad predictions regarding the possible extent and mechanism of effects in living organisms (see Chapter 4).

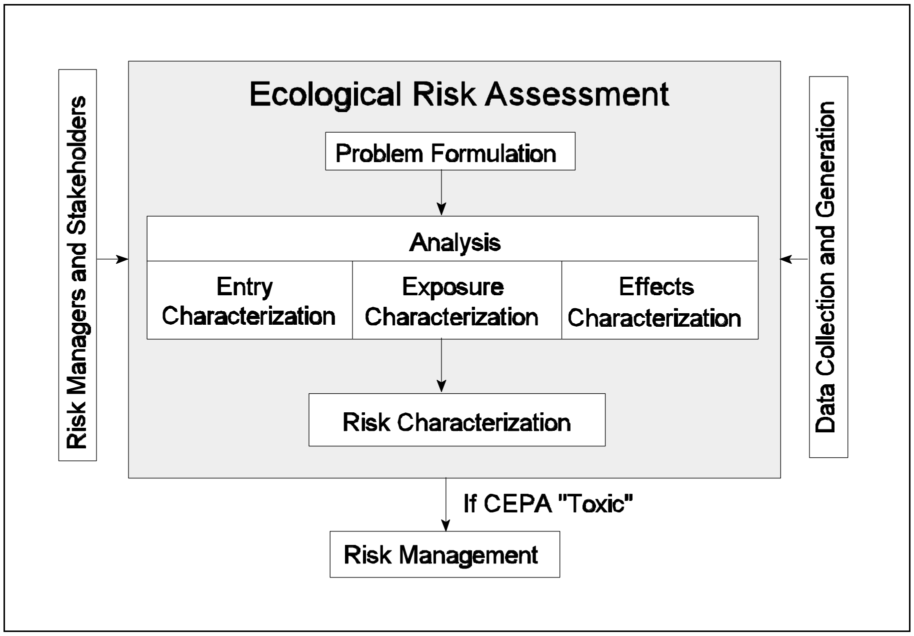


Figure 1 The risk assessment process as proposed for use under CEPA (Environment Canada, 1997)

Ecological risk assessment involves estimating the concentration of potentially deleterious substances (i.e., the exposure) experienced by specific biological populations. This concentration is either measured directly, or estimated from amounts of input from a known source and subsequent redistribution between various environmental compartments. The exposure may then be compared with previously demonstrated exposure-response relationships leading to some toxicological endpoint. Overall, the risk to a population associated with pulp mill effluents will vary according to four factors:

- Physical and chemical properties of componds in the effluent;
- Input concentrations and rates;
- Environmental redistribution, and
- Environmental concentration-response relationships.

There has been considerable progress since 1993 in characterizing more completely ClO_2-bleaching effluent compounds (Chapter 2). It is also possible to predict, with a quantifiable degree of certainty, the environmental redistribution from physicochemical and environmental properties (e.g., through fugacity-type or other partitioning models). There is, however, insufficient knowledge of concentration-response relationships at the sub-organismic, population, community or ecosystem level (see Chapters 4 and 5) for some of the recently elucidated compounds. It is clear that recent research on biological effects of treated pulp mill effluent has undergone a switch in emphasis with regard to contaminants of concern, to compounds more directly associated with the pulping as opposed to bleaching process. Some of the contaminants of concern are also natural plant products, or may be produced naturally in the aquatic environment.

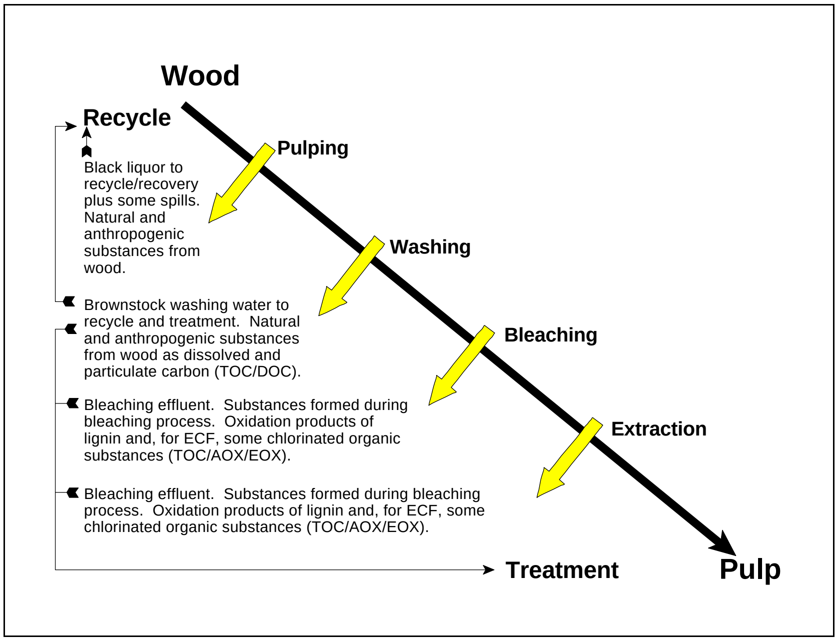
2 CHARACTERIZATION OF POTENTIALLY HAZARDOUS SUBSTANCES

2.1 FACTORS INFLUENCING THE PRODUCTION OF ORGANIC SUBSTANCES IN PULP MILL EFFLUENT

Organic material is present in effluent from the manufacture of all types of pulp. Various sum parameters are commonly used to measure the quantity of material present because the procedures involved in their determination are simple and can be performed routinely without elaborate equipment. Sum parameters are valuable for monitoring purposes within mills and provide a partial indication of the type of material present, but do not provide any information about the individual substances present. The determination of individual substances is much more complicated and is discussed in Section 2.3. Since environmental effects are often associated with specific substances or groups of substances, they should not be attributed to levels of sum parameters. Common sum parameters used to assess effluent characteristics are described below.

Total Organic Carbon (TOC) is a measure of the amount of all organic substances in effluent. Chemical Oxygen Demand (COD) is a measure of the oxygen consumed in the chemical oxidation of organic material in effluent by a strong chemical oxidant. TOC and COD are general sum parameters which can be used on effluent from all stages and types of pulp production. Effluents also contain chlorinated substances which are produced during the bleaching of chemical pulp as shown in **Figure 2**. Chlorinated organic substances can be measured by the sum parameter, adsorbable organic halogen (AOX). Extractable organic halogen (EOX) is a less commonly used sum parameter which refers to the amount of halogenated organic material which can be extracted from the effluent by a very non-polar solvent.

By the very nature of the test, AOX (or EOX) does not provide any information on the chemical species present and, with the mixture of compounds typically present in pulp mill effluent, AOX does not correlate with persistence or bioaccumulation. The use of this measurement for regulatory purposes also ignores the fact that the toxicity and biological properties of the organochlorine substances vary widely; the lower chlorinated substances generally presenting less risk than those with a higher degree of substitution (Solomon *et al*., 1993). Several studies have shown that AOX concentration is not related to biological responses (NCASI, 1990; O'Connor *et al.*, 1993*;* Robinson *et al.*, 1994).



Black liquor to recycle/recovery plus some spills. Natural and anthropogenic substances from wood.

Brownstock washing water to recycle and treatment. Natural and anthropogenic substances from wood as dissolved and particulate carbon (TOC/DOC).

Bleaching effluent. Substances formed during bleaching process. Oxidation products of lignin and, for ECF, some chlorinated organic substances (TOC/AOX/EOX).

Bleaching effluent. Substances formed during bleaching process. Oxidation products of lignin and, for ECF, some chlorinated organic substances (TOC/AOX/EOX).

Figure 2 The process of pulp production and bleaching.

2.1.1 Influence of feedstock

The amount of organic material in pulp mill effluent from bleaching is determined by the lignin content and amount of organic material entrained in the brownstock arriving at the bleach plant. Prebleaching steps, such as extended delignification and oxygen prebleaching decrease the lignin content of the brownstock. These processes, and improved pulp washing, decrease the organic load of the material entering the bleach plant which, in turn, decreases the consumption of bleaching chemicals and the amount of organic byproducts produced during bleaching. Chlorinated organic substances result mainly from reaction of residual lignin in pulp with chlorinating agents. Extended delignification and oxygen prebleaching therefore also decreases the amount of chlorinated substances formed during bleaching. Since the lignin content of hardwood pulps is less than softwoods, effluents from the bleaching of hardwoods contain smaller amounts of chlorinated substances.

2.1.2 Other controlling factors

It is well established that concentrations of chlorinated organic substances (as AOX) in effluent from ECF (elemental chlorine free) bleaching are five to ten times lower and different in composition from those of chlorine-based effluents (Solomon *et al*., 1993). As discussed in the previous assessment, chlorine dioxide acts primarily as an oxidant during bleaching. The only chlorinated material formed results from reaction of the byproduct hypochlorous acid with organic material.

2.2 NATURALLY-OCCURRING ORGANOCHLORINES IN SOFTWOOD AND THE ENVIRONMENT

To obtain a perspective of any possible environmental impact of pulp mill organochlorines, and, given the rapid increase of information within this field, the structure, formation, and sources of natural chlorinated material must be considered.

Several recent original papers and reviews have stressed the considerable concentrations of chlorinated organic material occurring as natural background levels in receiving waters (Grimvall, 1995; Grimvall *et al.*, 1994; Gribble, 1994; 1996; Dahlman *et al.*, 1993a; 1993b, Moore, 1995). These background concentrations show considerable area-specific variations, which must be accounted for when assessing environmental risks from the pulp industry. At the time when bleaching with chlorine came into the political and environmental spotlight, natural background concentrations of organochlorine compounds were not considered, leading to assumptions of large-scale contamination of the environment by organochlorines discharged from the pulp and paper industry. These assumptions were based on measures of AOX and EOX from receiving waters. Recently, some 2000 chlorinated and other halogenated compounds had been identified as entering the environment via synthesis in plants, marine organisms, bacteria, insects, fungi and mammals (Gribble, 1994). Abiotic processes such as active volcanoes, forest fires, chemical reactions in the atmosphere etc., further contribute to the formation of organochlorine compounds within the biosphere.

The range of naturally produced compounds includes both simple alkanes such as chloromethane, as well as numerous complex halogenated alcohols, ketones, carboxylic acids, carboxylic amides, aldehydes, epoxides and alkenes (Gribble, 1994 and references therein). For example, it has been estimated that the global emission rate of chloromethane from terrestrial biomass is 5 million tons per year, whereas the anthropogenic input is only 26,000 tons (Gribble, 1994). Other identified organochlorine compounds include a large number of chlorinated terpenes and related compounds that have been isolated from terrestrial and marine organisms, chlorinated fatty acids, chlorinated prostaglandins and chlorinated lipids (Gribble, 1994). Gribble (1996) reports that there are now 2400 natural halogenated substances identified.

When considering the products of ECF bleaching of pulp, it is important to recognize that many of the chlorinated substances produced in this process are also formed in nature. Several chlorophenolic isomers with biogenic origin have been isolated. Studies in Sweden (Grimvall *et al.*, 1994) have demonstrated that 2,4,6-trichlorophenol and its methylated analogue 2,4,6-trichloroanisole are ubiquitous in humus-rich waters and are formed by the action of microorganisms. Some degradation products from natural chlorinated lignin that are similar to those occurring in bleached kraft mill effluents are shown in **Figure 3** (Dahlman *et al*., 1993a).

The formation of chlorinated substances by organisms is catalyzed by commonly occurring enzymes. The occurrence of haloperoxidase enzymes seems to be the requirement for marine organisms, terrestrial plants, fungi, bacteria and mammals to synthesize halogenated compounds in the presence of chloride, bromide or iodide ions. Interestingly, human white blood cells contain myeloperoxidase, which, in the presence of chloride and other halogen ions and hydrogen peroxide, rapidly forms reactive halogens to destroy invading microorganisms. These reactions can extend to the formation of more persistent and bioaccumulative substances from simpler substances. An illustration of this is the enzymatic conversion of chlorophenols into PCDDs and PCDFs by horse-radish peroxidase enzyme (HRP) (Gribble, 1994).

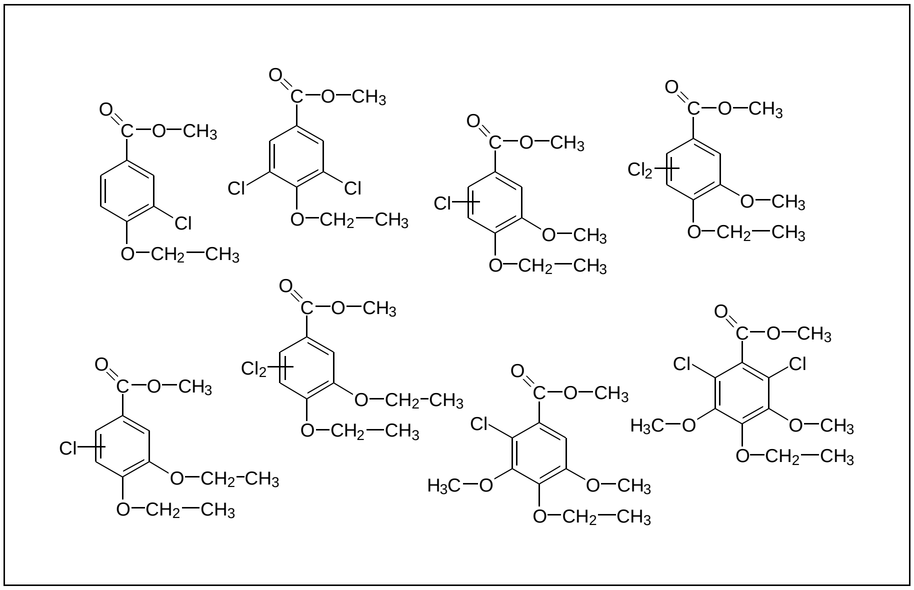


Figure 3 Chlorinated aromatic compounds identified from samples of degraded fulvic acid from both natural and bleached kraft mill effluents (Redrawn from Dahlman *et al.*, 1993a).

A natural consequence of the formation of chlorinated organic compounds is that there also must be pathways for their biodegradation and that these have been selected for and evolved over many eons. Evidence for these degradative processes includes the direct measurement of the degradation of specific substances as well as inferred degradation through indirect evidence based on measurements of organically bound chlorine in watersheds (Gribble, 1994; Landner *et al.*, 1994). Simple organochlorines such as di- and tri-chloromethane are degraded to carbon dioxide and water in soil. The chloride ion will dissolve in water and leach into ground or surface waters or be incorporated into new microbiologically synthesized natural chlorinated organic products. Also, chlorophenols are degraded by fungi. A white-rot fungus is known to degrade pentachlorophenol, DDT, and PCBs (Gribble, 1994). Studies on fluxes of organically bound chlorine in Lake Vättern in Sweden showed lower net export than import despite the fact that a pulp mill producing bleached pulp has been in operation on the lake since the 1950s (Landner *et al.*, 1994). As shown by Archibald *et al.* (1997), both naturally occurring AOX, as well as pulp mill related ECF AOX, is photomineralized in lake water. Lake Vättern is a clear water lake with a relatively deep photic layer and thus AOX will be subjected to extensive photooxidation in the summer time.

As also previously noted, terrestrial plants by themselves also are capable of synthesizing chlorinated compounds and may contribute to the occurrence of chlorinated substances in soils. Swedish studies (Asplund, 1992 and references therein) have shown that Norway spruce needles as well as the leaves from common beech contained 20-60 and 10-20 mg/kg (dry weight) organohalogens respectively. These results indirectly imply either that halogenation of organic compounds is occurring in trees used for pulp production or that these are present as a result of long-range transport of these substances from other, more distant, sources. The precise nature of the compounds included in the analysis of total organic halogens (TOX) remains unclear. A recent study by Johansson *et al*., 1996 (in manuscript) showed that fresh wood parts of spruce contained 10 µg/g d.w. organochlorine compounds. As to the origin of chlorinated organic substances in wood, it is unlikely that these types of high molecular weight compounds would originate from long-range transport of chlorinated substances as they are not characteristic of those found in precipitation as identified by Dahlman (pers. comm.) and Grimvall *et al*. (1994). Wood attacked by fungi contains higher concentrations of chlorinated substances than fresh healthy wood. Thus, storage conditions as well as the age of the trees may cause differences in the background concentrations of chlorinated substances entering the pulping process. The contribution of such material to the chlorinated organochlorine content in bleached kraft mill effluents (BKMEs) is unclear but would be less than 0.1 kg/tonne of pulp. In general terms, zero AOX concentrations will not occur, even in effluents from TCF production, since chloride ions from the wood furnish will always be present enabling some microbial formation of AOX.

It was shown by Johansson *et al.* (in manuscript) that the TOX-value increased along with increasing levels of chlorinated methyl esters in decaying spruce and in the humic layer in a spruce forest. This suggests that decomposors were synthesizing low molecular chlorinated compounds, possibly by using lignin as the carbon source for the chlorination. The TOX:organic matter ratio has been found to be more than ten times higher in soil than in fresh plant material. This halogenated material will at least partly be introduced via land run-off water to watercourses. Asplund (1992) showed that the concentration of chlorinated organic substances in surface waters was correlated to humic acid content. High AOX values were primarily found in humus-rich and oligotrophic lakes. Ongoing research (Lehtinen *et al.* unpublished) has confirmed the presence of considerable concentrations of AOX in peat bog water (130 µg/L). In comparison, an ECF effluent with an AOX discharge of 0.5 kg/adt and a 100 m^3/t water consumption with a receiving water dilution of 100:1 would reach an AOX concentration of 50 µg/L.

The great similarity between the chlorolignin of ECF pulp mills and natural chlorolignin, raises the question of which process is producing more AOX, production of pulp or natural processes. For Lake Vättern in Sweden, it was found that 25% of the input of AOX was of natural origin and 60% of the total AOX was degraded. Only 10% of the total input was retained in the sediment despite a 50-year contribution from two pulp mills. The recent work by Archibald *et al*. (1997) provides evidence that biotreated pulp mill AOX was, to a large extent, photomineralized over a relatively short, 4 month period, supporting the conclusions made for Lake Vättern that AOX from pulp mills is not recalcitrant in nature.

Chlorinated organic compounds are synthesized and degraded in the environment by natural biological and chemical process. This natural production varies, depending on the geographical location. Organisms have evolved in environments with background concentrations of natural chlorinated organic compounds. Many *compounds identical, or similar to those formed during ECF bleaching of pulp, are produced by natural processes. There is evidence that organisms possess mechanisms for effective breakdown of these types of chlorinated substances. Thus, chlorinated compounds formed during ECF/TCF pulp production technology, will neither be recalcitrant with respect to breakdown in the environment nor resistant to biodegradation. Pulp mill AOX will ultimately be mineralized through photochemical and biological processes. During this mineralization, the chlorinated organic material will be released as chloride ions and CO_2.*

2.3 PROGRESS IN THE CHEMICAL CHARACTERIZATION OF PULP MILL EFFLUENT

The chemical characterization of pulp mill effluent involves both the identification and quantification of individual compounds present. The process is much more difficult and time-consuming than that of a more general characterization using sum parameters because effluents contain very complicated mixtures of hundreds of different compounds. In addition, changes in bleaching and other pulp processing technologies result in the formation of different types and amounts of chemical byproducts. Fortunately, standard analytical methods developed for important groups of compounds, e.g., PCDDs, PCDFs, chlorophenols, resin and fatty acids, and chloroform, can be applied to any type of effluent. Other, more general analytical procedures have also been used recently to analyze effluents from mills employing ECF bleaching, with the result that a significant amount of information has been obtained since the 1993 review (Solomon *et al*., 1993) concerning the nature of the compounds present, particularly in bleaching effluent. Less is known about compounds present in effluents from mills employing TCF bleaching because the use of chlorine-free bleaching agents (and therefore the absence of chlorinated byproducts) has been equated with greater environmental benefit. However, it is clear from recent literature discussed in later sections of this document that environmental effects observed in fish are not related to the type of bleaching used. Some information is available on certain groups of compounds, such as chlorinated hydrocarbons (Section 2.3.2) and sterols (Cook *et al*., 1997) in final, biotreated effluent from mills employing ECF bleaching.

2.3.1 Products produced by processes other than bleaching

Pulping is the separation and conversion of wood fibers to a “pulp” by physical and/or chemical processes. The kraft process, which involves cooking the wood fibers with a solution of sodium hydroxide and sodium sulfide, is the main process for producing chemical pulp. The resultant pulping (black) liquor contains degraded cellulose, hemicellulose and lignin, and extractives, the specific components of which are not normally investigated since they are combusted during chemical recovery. As a result, no significant new information has been obtained since the 1993 review concerning products produced during pulping. Extended delignification and improved pulp washing increase the amount of organic material retained in pulping liquors. Oxygen delignification further decreases the lignin content of the unbleached pulp. Since effluent from oxygen delignification is also recycled with the pulping liquors, the chemical components present also are not normally investigated. However, based on model compounds studied (Gierer, 1986) and the analysis of functional groups present in lignin after treatment with oxygen (Gellerstedt and Lindfors, 1987; Lachenal *et al.*, 1995), polar, water-soluble, oxygenated compounds such as carboxylic acids are likely present.

2.3.2 Products produced during bleaching

In the previous review, fundamental differences in the chemistry of chlorine and chlorine dioxide bleaching were discussed. Chlorine dioxide is a more powerful oxidant than chlorine, and is capable of oxidizing the aromatic phenolic rings of lignin beyond the quinone stage, characteristic of chlorine bleaching, to muconic acids (**Figure 4**). The only chlorinated products which are formed result from reaction of organic material with the byproduct hypochlorous acid, and not chlorine dioxide.

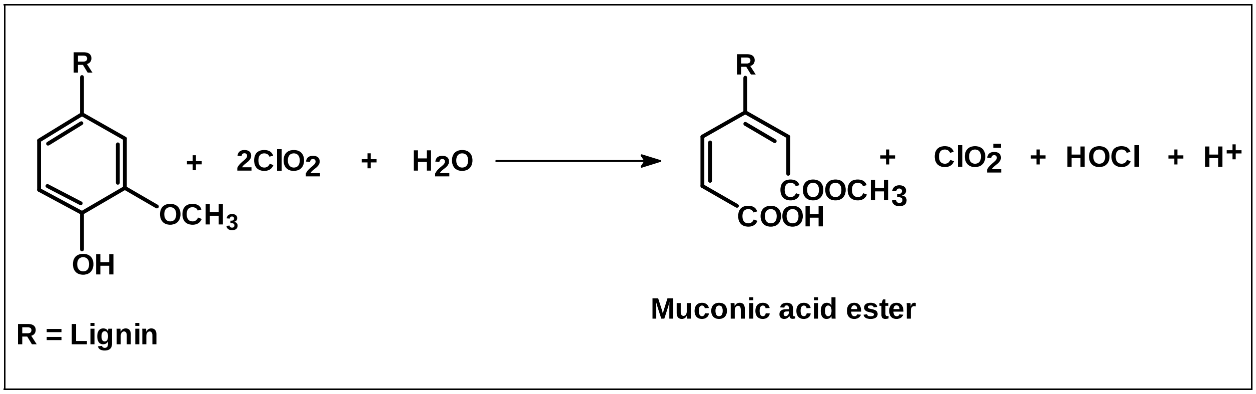


Figure 4 A reaction of lignin with chlorine dioxide

Approximately one hundred compounds have now been identified in effluents from ECF bleaching. Appendix 1 lists a number of these along with their structures. The total quantity of chlorinated material in ECF bleaching effluent is, however, only 10-20% of that found in chlorine-based bleaching effluent (Solomon *et al*., 1993). Most (nearly 60%) of the chlorinated compounds fall into two chemical classes - chlorophenols and chlorinated hydrocarbons. The remainder fall into a variety of classes including acids, furanones, aldehydes and ketones. A special effort has been made to monitor formation of PCDDs, PCDFs and chlorinated phenols. In the previous assessment, it was reported that levels of some compounds produced during bleaching, particularly PCDDs, PCDFs, polychlorophenols and chloroform, decreased dramatically when chlorine dioxide was substituted for chlorine in the first stage of bleaching. Numerous reports have now firmly established that ECF bleaching reduces levels of PCDDs, PCDFs and polychlorophenols in bleaching effluent to below current detection and/or proposed EPA regulatory limits (Federal Register, 1996; Luthe and Berry, 1997; Shariff *et al.*, 1996; NCASI, 1995). These results have commonly been achieved using pulp produced by conventional pulping, without extended delignification or prebleaching.

As reported in the 1993 assessment, the monochlorophenol, 6-chlorovanillin, is the main chlorophenol present in effluent from softwood bleaching. Traces of mono- and dichlorosyringaldehydes are present in hardwood bleaching effluent, particularly from eucalypt pulp (Smith *et al*., 1995). The chlorinated hydrocarbons were reported in recent work from Finland. Koistinen *et al*. (1994) reported that final biotreated effluents from the ECF bleaching of birch pulp contained ng/L (parts per trillion) concentrations of alkylchlorophenanthrenes (including chlororetenes - see **Figure 5**) and alkylchloronaphthalenes. The authors suggested that aromatic precursors, such as phenanthrene, may be present in oil-based defoamers as well as the unbleached pulp. Rantio (1995; 1996) reported that final biotreated effluent from both softwood and hardwood bleaching contained traces of several isomeric chlorocymenes. Concentrations decreased dramatically compared to those present when chlorine was used for bleaching. Other substances recently identified in ECF bleaching effluent include muconic acid esters derived from guaiacol (Vilen *et al*., 1996), dichloromethylenefuranones (McKague and Grey, 1996), 4-chloro-3-hydroxy-2H-pyran-2-one (Smith *et al*., 1994), dichlorocyclohexenediones (McKague and Grey, 1996) and straight chain aldehydes and ketones (Juuti *et al*., 1996). These other new compounds are non-aromatic (**Figure 5**) and contain functional groups which render them susceptible to degradation in the environment (Schwarzenbach *et al*., 1993).

Forty to fifty percent of the material in ECF bleaching effluent has a molecular weight > 1000 (McKague and Carlberg, 1996). Studies performed on this material have shown it is highly polar and characterized by a low (<2%) chlorine content. The aromatic content is lower in material from hardwood bleaching (Dahlman *et al*., 1995) and oxidative degradation gives similar products to those obtained from naturally occurring humic substances (Dahlman *et al*., 1993a). The latter finding suggests humic substances and the high molecular weight material from ECF bleaching have macromolecular structural characteristics in common and that this material from bleaching is harmless in the environment. Carbohydrates, mainly xylan structures, may account for nearly 30% of the high molecular weight material (Dahlman *et al*., 1995; Wallis and Wearne, 1994).

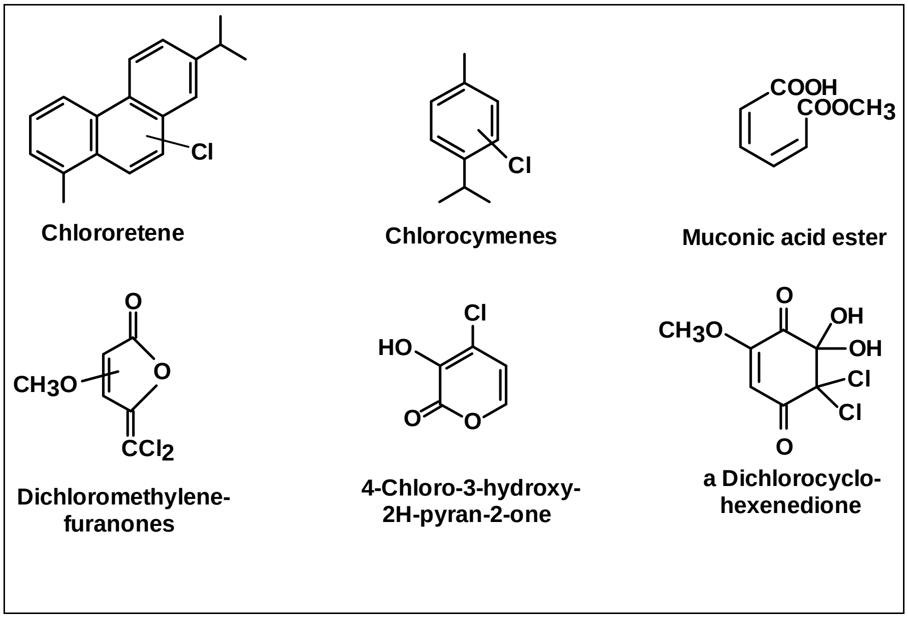


Figure 5 New organic substances identified in effluent from ECF bleaching

TCF (totally chlorine free) bleaching involves a sequence of stages which may include oxygen delignification, enzyme pretreatment, chelation to remove transition metals, alkaline hydrogen peroxide, other peroxide stages, ozone and acid washing. Oxygen reacts in a similar manner to chlorine dioxide with phenolic structures in residual lignin, resulting in the formation of ring-opened carboxylic acids (Gierer, 1986; Lachenal *et al*., 1995; McDonough, 1996). Hydrogen peroxide mainly destroys carbonyl groups introduced by previous bleaching steps, unless elevated temperatures are used, in which case it also acts as a delignifying agent (Lachenal, 1996). Ozone, like chlorine, reacts with aromatic and olefinic groups present in lignin, but, unlike chlorine, introduces large numbers of carboxyl groups by breaking the ring structure as shown in **Figure 6**.

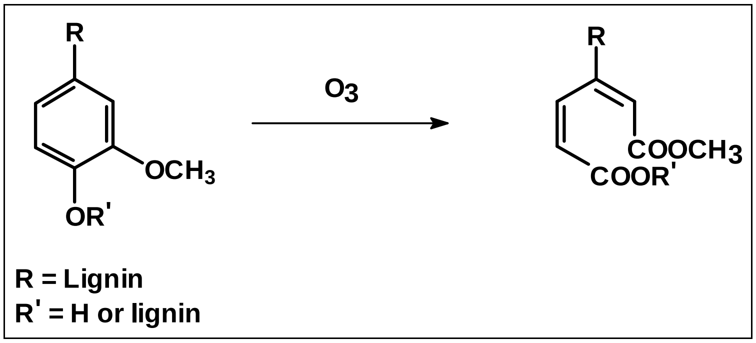


Figure 6 The reaction of lignin with ozone

Characterization of the substances in effluents from TCF bleaching has received little attention. However, effluents from ozone bleaching contain a large number of aliphatic mono- and dicarboxylic acids (Sonnenberg *et al*., 1992; Soteland *et al*., 1994). Formic, acetic, oxalic and glyoxylic acid, glyoxal and vanillin are major low molecular compounds formed. TCF bleaching effluents also contain chelating agents used to remove transition metals prior to hydrogen peroxide bleaching (Lapierre *et al*., 1995). The proportion of high molecular weight material in TCF bleaching effluent may be similar to or slightly lower than that of ECF effluent, although more information is needed (Dahlman *et al*., 1995).

Individual substances present in effluents from current pulping and prebleaching processes are not normally investigated since they are recycled during chemical recovery. Numerous studies have firmly established that concentrations of PCDDs, PDCFs and polychlorophenols in effluents from ECF bleaching are close to or below the level of detection. Substances identified in ECF bleaching effluent since 1993 are predicted to be readily biodegradable whereas little is known about the types of compounds in TCF bleaching effluent.

2.4 NATURE OF SUBSTANCES DISCHARGED IN FINAL, TREATED EFFLUENT

Pulp mill process streams are usually combined and treated in aerated lagoons or activated sludge systems prior to final discharge. Low molecular weight compounds are degraded by biological and chemical processes while abiotic processes are responsible for the degradation of high molecular weight material. The more highly oxidized products produced during treatment are more water soluble and less likely to bioaccumulate in aquatic organisms. Occasionally, because of local anaerobic or anoxic conditions within biotreatment systems, compounds which are normally biodegradable may be poorly degraded or undergo biotransformation into other products which are more lipophilic and have a greater potential for bioaccumulation. The previous assessment discussed the biological methylation/demethylation of chlorinated catechols, guaiacols, and veratroles. Resin acids have been reported to undergo biological hydrogenation/ dehydrogenation and/or decarboxylation to neutral diterpenoids such as dehydroabietin, retene and fichtelite as well as the expected oxidation products (Wilkins and Panadam, 1987; Zender *et al*., 1994; Stuthridge and Tavendale, 1996). The resultant products may be more resistant to biodegradation, particularly if they are aromatic. In a study of removal efficiencies of sterols, the concentration of stigmasterol was found to increase during biotreatment (Cook *et al*., 1997). Resin acids and sterols are constituents of pulping effluent which may be carried over to the bleach plant with the pulp because of their lipophilic properties so are not related to ECF bleaching.

Final mill effluent from mills with ECF bleaching may contain traces of halogenated compounds. For example, chlorinated acetic acids, chloroform and chlorinated resin acids have been reported in ECF effluent after biological treatment (Dahlman *et al*., 1993b). 1,1-Dichlorodimethyl sulfone is known to be highly resistant to biodegradation and appears in final effluent. A study using muconic model compounds showed biodegradation of these types of compounds was dependent on the chemical structure of the substrate (Zheng and Allen, 1996). The high molecular weight material formed during bleaching undergoes little biodegradation, however, as mentioned in section 2.3.2, it has been shown to resemble natural aquatic humic material (Dahlman *et al*., 1993a) and undergo gradual abiotic degradation in the environment (Roy-Arcand and Archibald, 1993).

Low concentrations of halogenated compounds may also continue to be released from lagoon sediment after conversion to ECF bleaching. In one study, it was estimated that depletion of historic deposits of 2,3,7,8-TCDF from an aerated lagoon sediment at a bleached softwood pulp mill would require one to four years (Pagoria and Kerfoot, 1997). A variety of studies have shown sediments continue to provide a source of chlorophenols (Kvernheim *et al*., 1993; Palm and Lammi, 1995; Judd *et al*., 1995). The trace levels of chlorinated hydrocarbons reported in final biotreated effluent after conversion to ECF bleaching (Section 2.3.2) may also originate from historical deposits in lagoon sediment. As mentioned in the 1993 review, chlorate is removed during biotreatment.

Substances produced during treatment of effluent are normally highly oxidized and do not bioaccumulate. Occasionally, products such as retene may be formed as a result of anaerobic transformations. Traces of chlorinated substances released in final biotreated effluent may originate from the bleaching process or from pre-ECF bleaching deposits in lagoon sediment.

3 ENVIRONMENTAL FATE PROCESSES

The possible effects of substances in the environment are dependent on the properties of the substances themselves and processes that affect the exposure of biota. The following sections provide an update on -

- The range of physicochemical properties of compounds previously described in pulp mill effluent;
- The biogeochemical processes that influence the environmental behavior of effluent substances;
- Processes that influence the longevity of effluent constituents released to the environment such as microbial degradation and metabolic modification; and
- Environmental studies of the observed environmental behavior and changes in concentration of pulp mill constituents.

3.1 PHYSICOCHEMICAL PROPERTIES OF PULPING/ClO_2 BLEACHING BYPRODUCTS

Chapter 2 discusses the types of compounds that have been found in pulp mill effluent, and reviews recent studies on naturally-occurring chlorinated organic compounds as well. The movement of contaminants through an ecosystem is controlled by the physicochemical properties of individual compounds, as well as the characteristics of various environmental compartments. The partitioning of a substance among various compartments such as particulates, sediment, water, or air will in turn control the net concentrations and availability for partitioning into the tissues of living organisms.

The potential for entry of a substance into or adsorption onto a target organism is a necessary condition for expression of a direct adverse effect. From a risk assessment perspective, an exposure pathway is only operative if a substance can pass from an environmental compartment to a living organism (**Figure 7**). The bioavailability of a substance may be defined as the potential for entry of a substance into an organism – a process termed bioaccumulation. Bioconcentration occurs when an organism assimilates a substance at a concentration in excess of the concentration in the immediate environmental compartment. Biomagnification is defined as the progressive increase in tissue concentration of a substance with increasing trophic position in different organisms within a food web, i.e., the concentration in a predator is higher than in its prey.

Properties of compounds from pulpmills that are relevant to environmental partitioning include molecular size, polarity, and specific structure. These chemical properties affect the hydrophobicity or aqueous solubility, vapor pressure, octanol-water partition coefficient (K_{OW}), and the organic carbon partition coefficient (K_{OC}) of a substance. In addition, Henry's Law constant (H_C) provides an estimate of the partitioning of a substance between air and water. Henry's Law constant can be estimated as the ratio of vapor pressure to aqueous solubility.

The partition coefficient of a substance between octanol and water (K_{OW}) is a measurement (or, sometimes, estimate) of the tendency to partition into a non-polar medium, as opposed to water.

Since all cells are surrounded with lipid membrane, and many organisms use lipids as an energy store, K_{OW} is also a useful index of the potential for:1) bioaccumulation, 2) bioconcentration, and 3) biomagnification. Substantial bioaccumulation does not generally occur for compounds with a $K_{OW} < 2000$ (or $\log K_{OW} < 3.3$). Highly bioaccumulative substances, which tend to biomagnify through the food chain, generally exhibit a $\log K_{OW} > 5$ (Mackay, 1995).

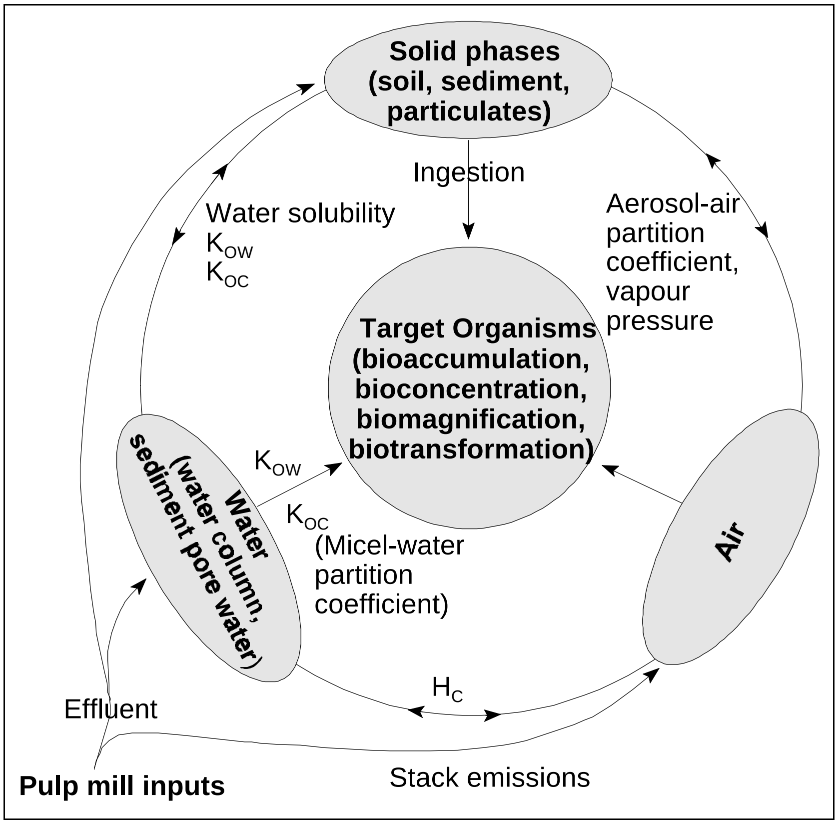


Figure 7 Diagrammatic representation of exposure pathways in pulp mill effluents.

Substances that do not tend to readily bioaccumulate or bioconcentrate may, nonetheless, be taken up by biota, and exert deleterious effects. The expected toxicological effects of poorly bioaccumulated but potentially deleterious substances include acute lethality or profound declines in growth or fitness in individuals directly exposed. Where exposures to these substances are continuous, such as in an effluent stream from a continuously producing factory, responses in organisms may be similar to those for bioaccumulative and persistent substances — in either case, the organisms experience continuous exposure. Biological responses in other species outside of the immediate area of contamination, and that are typically mediated by transfer of contaminants between organisms within a food web, would not be expected.

Lower molecular weight, hydrophilic substances tend to rapidly come to equilibrium based on physical partitioning in the basence of biological mediation. Also, contaminants with lower molecular weights tend to be more readily modified, either through biodegradation or biotransformation, are generally less persistent, although molecular weight is not entirely synonymous with expected environmental longevity. Furthermore, hydrophilic contaminants do not cause the unanticipated effects that have occasionally been encountered in association with hydrophobic and persistent organic contaminants that biomagnify, such as dioxins and furans.

The aqueous solubility of a substance is a measure of its tendency to occur in dissolved form. Substances with a very high $\log K_{OW}$ and extremely low aqueous solubility tend to remain tightly sorbed to soils and sediments, and are not readily dissolved and transported in water, except through the bulk movement of particulates.

Organic carbon-water partitioning coefficient (K_{OC}) values are expected to be correlated with K_{OW} values across groups of individual organic contaminants, since K_{OW} and K_{OC} are both strongly influenced by the polarity and size of a molecule; however, reliable estimates of K_{OC} are not available for many of the compounds found in treated effluents from ECF mills. Closely related to K_{OW} and K_{OC} is the partition coefficient of a substance between surface sediment and the water column in aquatic systems, sometimes referred to as “K_D” (Schindler *et al*., 1995). K_D values have typically been calculated from field data, in a limited number of studies.

Chlorinated organic contaminants exhibit a range in their tendency to volatilize or evaporate, and partition into air from sources in soil, other solid material, or water. Henry's Law constant values, the tendency to partition from water to air, are also correlated with the relative tendency for partitioning from water-saturated soils into air. In general, more highly-chlorinated congeners (variants of a type of compound) are less volatile and less soluble in aqueous solutions. More highly chlorinated congeners, therefore, have less tendency to move through the environment, and tend to remain isolated in sediment repositories, i.e., geological “sinks”. More highly chlorinated congeners are also more likely to be distributed closer to their original source than their lower-chlorinated counterparts. It is important to note, however, that the relationship between degree of chlorination and other properties such as hydrophobicity, volatility, or resistance to degradation applies only to structurally similar compounds, e.g., across different dioxin or chlorophenol congeners.

Table 3.1 categorizes the known constituents of effluent from ECF pulp mills according to expected molecular size, physicochemical properties or octanol-water partition coefficient, and expected route of exposure for aquatic organisms. Although partition coefficients and various physicochemical properties for many of the hundreds of compounds in ClO_2-bleaching pulp mill effluent have not been determined, reasonable estimates of chemical properties can be estimated through comparison with structurally related compounds. In Table 3.1, the properties of the molecular group as a whole have been extrapolated from a handful of constituent compounds in each group (shown in bold) which have previously been examined in more detail.

As noted in Section 1, the assessment of risk associated specifically with the use of ClO_2 in bleaching is related to but may be considered a distinct activity from the assessment of risk associated with environmental inputs of ECF mill final effluents, where various constituents are contributing by other mill processes such as pulping.

Table 3.1 Compounds previously detected in pulp mill final effluents (before and after treatment) and expected environmental behavior

	1. Low molecular weight (MW) compounds	2. Intermediate MW[1] moderately polar, non-chlorinated compounds	3. Intermediate MW moderately polar chlorinated compounds	4. Intermediate MW non-polar, non-chlorinated compounds	5. Intermediate MW non-polar, chlorinated compounds	6. High MW[2] compounds
Examples	acetone, chlorate ion, chloroform, acetaldehyde, methyl ethyl ketone, dichloromethane, carbon tetrachloride, 1,2-dichloroethane, dichloroacetonitrile	**nonchlorinated phenols**, **catechols, guaiacols**, syringols, vanillins, hexanedienedioic acid monomethyl-ester,	**chlorophenols,** **chlorocatechols,** **chloroguaiacols,** chlorosyringols. chlorovanillins, chlorinated hydroxybutanoic acids, 2,3,4,4-tetrachloro-3-hydroxybutanoic acid, 3-methoxy-5-dichloromethylene-2(5H)-furanone	Resin and fatty acids, **naturally-occurring PAHs**, including retene; plant sterols; hexane; xylenes, α- and β-pinene;	**PCDDs/PCDFs[3],** chlorinated resin acids, chlorinated dibenzothiophenes, chlorinated thiophenes **chlorocymenes**[4], chlorocymenenes[4]. chloronaphthalenes, chlorophenanthrenes, 2,5-dichlorothiophene	lignin derivatives, complex carbohydrates
Log K_{OW} range (at least for compounds in the class for which K_{OW} has been determined)	<< 1	0.5 - 1.5	2 - 5	3 - 9	4 - 9	highly variable; tending to be less dependent on molecular size than on the number of attached polar functional groups
Aqueous solubility	Moderate to High	Moderate to High	Moderate to High	Extremely Low	Extremely Low	highly variable
Primary environmental compartment where found under equilibrium conditions	water column	dissolved and particulate organic matter, suspended and deposited sediment	dissolved and particulate organic matter, suspended and deposited sediment	dissolved and particulate organic matter, suspended and deposited sediment	dissolved and particulate organic matter, suspended and deposited sediment	dissolved and particulate organic matter, suspended and deposited sediment
Principle Exposure Pathway	Water (dissolved)	Water and sediment	Water and sediment	Sediment and particulates	Sediment and particulates; food-chain mediated	Sediment and particulates
Pathway of Entry into Organisms	Respiratory and other external epithelia	Respiratory and other external epithelia	Respiratory and other external epithelia	Diet; directly from surrounding media	Diet; directly from surrounding media	
Amenable to Biomagnification ?	No	Limited	Limited	Limited	Yes	
Expected Environmental Half Life (where known)	minutes to days	hours to days	days to weeks	days to years	years	highly variable depending on molecular structure
Primary Mechanism of Removal from Environment	Volatilization and Photolysis, Biodegradation, Chemical degradation	Biodegradation and Biotransformation	Biodegradation and Biotransformation	Biotransformation; Burial in sediments	Burial in sediment	

1. Molecular weight from ca. 100 to 1000 Daltons
2. Molecular weight > 1000 Daltons
3. 2,3,7,8-TCDD and 2,3,7,8-TCDF not present at concentrations exceeding analytical detection limits in most effluent samples from mills employing 100%ClO_2 substitution in 1995/96.
4. Substantially reduced following conversion from Cl_2 to ClO_2 bleaching (Rantio, 1995).

Considerable research has been conducted on the molecular size distribution of substances found in treated pulp mill effluent: McKague and Carlberg (1996) provide a review. Particular emphasis has been placed on the separation through ultrafiltration or size exclusion chromatography of the operationally-defined high molecular weight (MW) fraction, comprising all substances with a molecular weight greater than 1,000 Daltons from a low MW fraction (< 1,000 Daltons). ECF Bleach kraft mill effluent typically contains organic material in the MW range of approximately 50 to 20,000 Daltons, with the major fraction (> 50%) being larger than 1,000 Daltons.

Dahlman *et al*., (1995) determined that the MW > 1,000 Dalton fraction in hardwood extractives was mainly non-aromatic lignin-derived substances, and aromatic lignin structures in softwood effluent. The carbohydrate content after acid hydrolysis of model effluent from various treatments varied from 3.5 to 48% of the total mass. Finally, all samples exhibited substantial concentrations of carboxyl functional groups (2.4 to 3.6 mmol/g), and smaller concentrations of either methoxyl groups (0.7 to 1.8 mmol/g) or phenolic hydroxyl groups (n.d. to 1.8 mmol/g). The ionizable carboxyl groups, in particular, reduce the hydrophobicity and enhance the aqueous solubility of residual lignin building blocks in both the effluent and in the receiving environment. Dahlman *et al*. (1996) state, “In their chlorine content and hydrophilic and polyelectrolytic characteristics, the investigated HMWM (high molecular weight material) resembled aquatic humic substances”.

With regard to ecotoxicity, special attention has been paid to dioxins and furans, chlorinated phenolics, chlorinated and non-chlorinated resin acids, and steroid-like compounds: mostly low MW substances under this scheme. Accordingly, Table 3.1 above distinguishes between several classes of “intermediate-molecular weight” compounds (100<MW<1,000 Daltons). The current state of knowledge on the environmental fate and effects of organic contaminants dictates that the evaluation of the potential for risks associated with various effluent types increasingly will require a better distinction of effluent properties for the < 1,000 Dalton size fraction

Chlorophenols may be considered to be moderately polar compounds depending on the degree of chlorination (the log K_{OW} for 2-chlorophenol is 2.15, and for 2,4,6-trichlorophenol is 3.69) (Schüürmann *et al*., 1996); hence, both the bioavailability and toxicity may depend more on dissociation and ionization in aqueous media than on lipophilicity.

For chlorinated organic compounds that are otherwise similar in structure, the K_{OW} tends to increase with the degree of chlorination. The log K_{OW} for 1-chlorobenzodioxin, for example, is estimated to be 4.75, whereas log K_{OW} for octachlorobenzodioxin is estimated to be 8.2 (Shiu *et al*., 1988). Log K_{OW} for tetrachlorodibenzodioxins is, on average, 6.4. The log K_{OW} for furans varies from 6.2 on average for tetrachlorodibenzofurans to 8.78 for octachlorodibenzofuran. In general, an increase in the number of chlorines increases the K_{OW} by a factor of approximately 4, and reduces the aqueous solubility by a factor of about 5 (Mackay, 1995). Tam *et al*. (1994) noted an increase in log K_{OW} from guaiacol to tetrachloroguaiacol of about 3.0 $\log_{10}$ units, or a factor of about 5.6 for each additional chlorine substituent. Substitution with 100% ClO_2 has been shown to decrease the degree of chlorination; hence, decrease the potential for bioaccumulation.

The environmental fate of chlorinated organic compounds with vastly different molecular weights or structures cannot be usefully compared based on the degree of chlorination. For example, the ecotoxicological properties of chlorinated hydroxybutanoic acids versus chlorophenols cannot be usefully compared based on either the number of chlorines attached, or the ratio of the degree of chlorination to molecular weight. Schwantes and McDonough (1994) suggest, based on earlier studies by Voss *et al*. (1980), Kringstad *et al*. (1984), and Salkinoja-Salonen *et al*. (1991), that the "ratio of chlorine to carbon" for various fractions of bleached kraft effluents is "an environmentally significant parameter" and a predictor of toxicity. Whereas, a reasonable relationship undoubtedly existed prior to ~1990 (e.g., in comparing Cl_2 with high ClO_2 substitution bleached kraft mill effluent), the correlation between toxicity and chlorine-carbon ratio in final effluents of ECF or TCF mills has not been demonstrated. Chlorine:carbon ratios should not, therefore, be assumed to be a predictor of overall toxicity.

Molecular size, hydrophobicity and various chemical coefficients are measures that have proven predictive power in assessing the extent to which a substance is divided among various physical, chemical and biological compartments in aquatic environments. These measures also allow some prediction of the likelihood of biological effects based on bioaccumulation potential, exposure pathways, and environmental persistence. The specific molecular and conformational structure of individual compounds, however, is the final determinant of the potential for adverse effects. For example, the naturally-occurring tree sterol β-sitosterol may undergo microbial transformation in the environment to produce a hormone-like substance capable of inducing masculinization in some fish species (Kovacs *et al*., 1995; see also Chapter 4). Sitosterol (**Figure 5**) is one of more than a dozen sterols or triterpenoids that have been identified in extracts from laboratory bleached pulp (e.g., birch pulp: Björklund Jansson *et al*., 1995), and in treated effluents from 22 U.S. pulp mills (Cook *et al*., 1997).

Environmental assessment of pulp mill effluents before the late 1980s focused on minimizing relatively non-specific effects on aquatic biota such as acute toxicity, nutrient enrichment, oxygen depletion and smothering (e.g., Pearson, 1980). Elucidation and minimization through management of much more subtle toxicological effects that are strongly mediated by the strength and kinetics of binding to a specific molecular receptor within a target organism requires a detailed assessment of individual effluent compounds and their environmental transformation products, rather than measurement of broad chemical classes. This approach has already been employed in understanding and mitigating dioxin risks. The mechanism of toxic action of 2,3,7,8-TCDD, i.e., extremely strong binding with the cytosolic Ah receptor, requires a clear discrimination among different dioxin and furan congeners during monitoring and risk management.

High molecular weight, hydrophobic, chlorinated organic compounds exhibit substantially different environmental behavior and toxicological modes of action *than more hydrophilic chlorinated or non-chlorinated compounds. Lower molecular weight, more soluble substances exhibit virtually no tendency to biomagnify, and less potential for exerting insidious or food-web mediated toxic effects at the community and ecosystem level. Minimization of highly bioaccumulative and persistent substances in final effluent will limit possible environmental effects to immediate consequences of limited duration for organisms directly exposed, followed secondarily by ecological consequences at higher levels of biological organization. It is clear that 100%* ClO_2 *substitution drastically reduces the production of higher chlorinated, more bioaccumulative phenolics (3 chlorine substituents) in final effluents. Few compounds in treated ECF mill effluents exhibit a log KOW > 5 (the threshold for substantial biomagnification potential) or have the additional property of being relatively persistent in living organisms due to their resistance to metabolic modification.*

3.2 ENVIRONMENTAL BEHAVIOR INCLUDING REDISTRIBUTION AND PARTITIONING

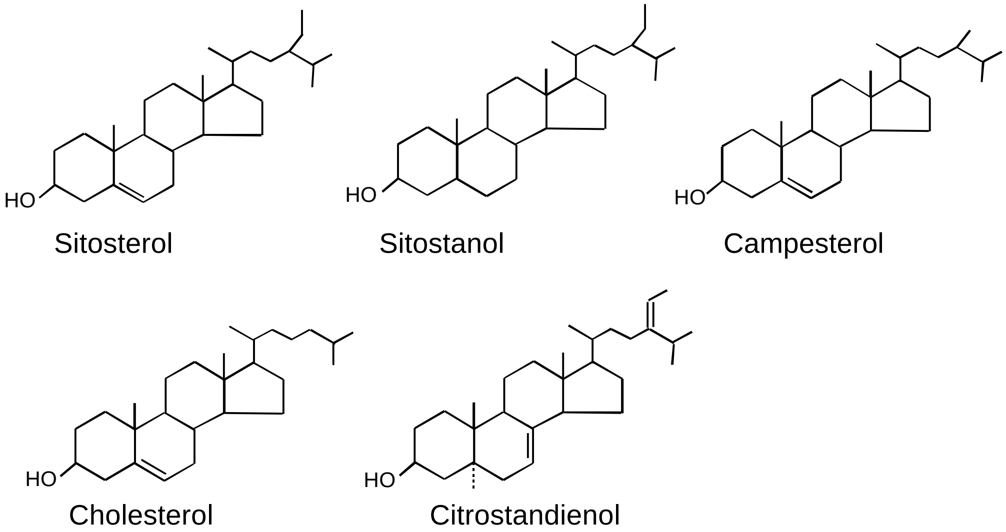
Partitioning coefficients, measured in a laboratory setting, have some general predictive power, especially in examining the relative fates of contaminants. These coefficients, however, have some obvious limitations when applied to prediction of fate in the field. One such limitation is the assumption that adjacent environmental compartments are in equilibrium with each other.

The occurrence in water of dissolved forms of substances containing organic carbon (dissolved organic carbon, DOC) or very fine colloidal materials (< 0.45 mm in diameter) can increase the apparent dissolved concentrations of high-molecular weight, non-polar compounds above levels predicted base on pure aqueous solubilities. The natural or background levels of DOC in marine or freshwater are directly influenced by the biological productivity of the system: the growth of plants and algae, and increase in the biomass of secondary and tertiary biological consumers lead to a greater concentration of excreted and detrital organic matter, including dissolved substances. In receiving waters for pulp mill effluent, the anthropogenically elevated DOC is expected to be a major factor in the partitioning of organic compounds between the aqueous phase as truly dissolved substances, water-borne particulates, surface sediment, and living organisms.

3.3 RELEASE OF NATURALLY OCCURRING SUBSTANCES FROM WOOD

Much of the research on the chemistry of softwood or hardwood pulping and bleaching has emphasized chemical reactions of cellulose, lignins and carbohydrate building blocks; however, virtually all higher plants contain hundreds of secondary metabolites including complex compounds such as di- and tri-terpenoids. Many of these compounds have been demonstrated to exert toxicological effects on various organisms. Indeed, the production of many of these compounds by plants is hypothesized to have evolved as a protective mechanism against insect pests, pathogens and herbivores. When trees are processed in a pulp and paper mill, many of the compounds released are the same as those released naturally, especially during decomposition of detrital plant material. Compounds released during plant decomposition, furthermore, are known to undergo secondary reactions once released to the environment.

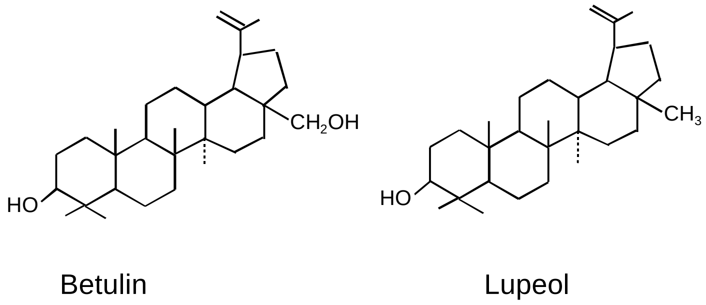
A) Examples of sterols identified in acetone extracts from oxygen bleached ECF pulp



Sitosterol Sitostanol Campesterol

Cholesterol Citrostandienol

B) Examples of triterpenoids identified in acetone extracts from oxygen bleached ECF pulp



Betulin Lupeol

Figure 8 Sterols and triterpenoids found in pulp mill effluent. (Adapted from Björklund Janson *et al*., 1995. For all compounds, sterol and triterpenoid concentrations decreased substantially after bleaching with chlorine dioxide.)

Studies of the environmental behavior, especially diagenesis (biogeochemical changes associated with aging and degradation), of detrital plant material are instructive in understanding the environmental behavior of some constituents of pulp mill effluent. A major difference between naturally released compounds and many of the constituents of pulp effluent released from a mill employing 100% ClO_2 substitution may be in the rates of input and concentrations produced, at least close to the mill.

Retene (1-methyl-7-isopropylphenanthrene) and tetrahydrochrysenes (THCs) are PAHs that are widely recognized to be of natural origin, produced from plant-derived diterpenoid and triterpenoid precursors (**Figure 9**; Bouloubassi and Saliot, 1993). Although these PAHs have been found routinely in marine sediment samples, they are probably formed primarily in terrestrial soils and/or freshwater environments and subsequently deposited to coastal areas, where sediment concentrations of retene or THCs were observed to be in the range 13-53 ng/g and 17 to 163 ng/g, respectively (Bouloubassi and Saliot, 1993). Retene is of particular interest with regard to the environmental assessment of pulp mill effluent, since retene has recently been demonstrated to be an MFO inducer (see Section 4). Other PAHs such as perylene have also been used as geochemical markers of continental inputs to the sea (Aizenschtat, 1973); perylene is derived from plant pigments under reducing conditions.

Johansson *et al*. (1995) demonstrated the presence of naturally-occurring non-chlorinated as well as mono-, di- and tri-chlorinated benzoic acids at concentrations from < 1 to 20 mg/g in peat, soil and decaying vegetation or trees. These are probably formed during the oxidative degradation of complex organic matter, and would also be expected to occur in ECF effluent, although little if any research has been conducted on this issue.

The pulping and bleaching of wood is not a prerequisite for leaching of potentially-deleterious substances into the aquatic environment (see also the discussion of naturally-occurring organochlorines - Section 2). For example, dissolved organic carbon (DOC), phenols, and resin acids were released from Scotch Pine (*Pinus sylvestris* L) or Norway spruce (*Picea abies*) when the pulpwood was sprayed with water, for the purposes of short-term preservation (Borga *et al*.,1996). DOC and phenol leakage occurred rapidly initially (within 8 to 14 days), and some re-absorption occurred thereafter. The actual concentration released was limited by microbial degradation in the storage system. The toxicity of the log storage wastewater, as determined by inhibition of bioluminescence in cultures of the bacterium, *Vibrio fischerii* (Microtox^{TM}) showed some relationship to DOC levels, but not obviously to phenol concentrations.

Overall, recent studies of the diagenesis of organic matter in terrestrial and aquatic environments underscore the importance of microbiologically-mediated transformation, including secondary production of complex organic compounds from natural plant products, as well as the degradation of complex polymers such as lignin.

Studies completed since 1993 suggest that some of the resin acids or naturally-occurring PAHs produced during the diagenesis of plant materials exhibit a similar environmental fate and effects as some of the compounds found in both ECF and TCF pulp mill effluent.

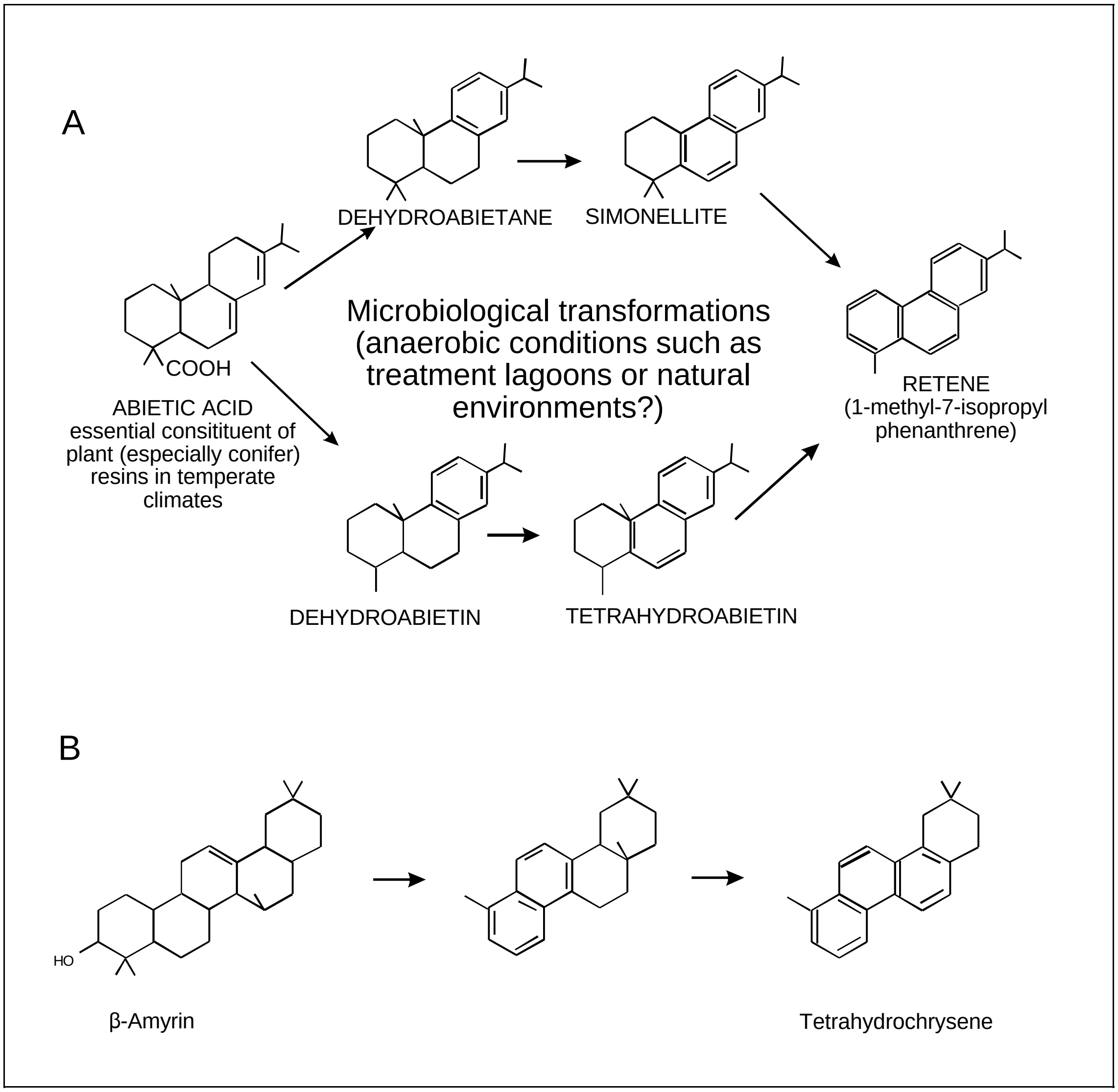


Figure 9 Biotransformation reactions in pulp mill effluent. (Adapted from Bouloubassi and Saliot, 1993).

3.4 DEGRADATION AND PERSISTENCE

The overall impact of any environmental input or perturbation is proportional not only to the concentration at a point in the ecosystem, but also to the spatial extent and longevity. Biodegradation of the constituents of pulp mill effluent once released to the environment is obviously important for long-term environmental effects. Processes that can reduce the total mass of a contaminant once released to the aqueous environment include: complete or partial destruction (including partial dechlorination) due to sunlight-mediated photolysis; microbial biodegradation; enzyme-mediated biotransformation within microbes and higher animals (especially vertebrates); and immobilization in geological sinks, such as deep burial in lake and ocean sediments.

In addition to mechanisms of environmental loss, some substances may increase in concentration as a result of transformations in the environment or during biological treatment from reactants in pulp mill effluent. The production of retene in sediments, both naturally and during treatment of pulp mill effluent is discussed above. Transformation products detected in pulp mill receiving environments and the processes that influence their production were discussed by Solomon *et al*. (1993).

3.4.1 Photolytic destruction

Loss mechanisms such as photolytic destruction or long-term burial in sediments are distinguished here from redistribution mechanisms such as dilution or volatilization at the air-water interface, which serve initially to redistribute the contaminant throughout a larger portion of the environment.

Generally, 2,3,7,8-TCDD and 2,3,7,8-TCDF are not detected in ECF effluent; however, the following brief discussion serves to highlight factors that influence environmental persistence from historical releases. Photolytic destruction of the highly persistent dioxins and furans has been studied to a greater extent in soils than in major aquatic compartments such as sediment or water, where light availability is strongly limited by the depth and turbidity of the overlying water. A limited portion of dioxins and furans contained in surface soils will disappear over extended periods of time, and half-lives for loss of dioxins/furans are reported to be in the order of six months to many years (Nash and Beall, 1980). Paustenbach *et al*. (1992) provided a review of the literature, and estimated the half-life of 2,3,7,8-tetrachlorodibenzodioxin in surface soil to be in the range 9 to 15 years. Dioxins/furans contained in the top 0.1 to 1 cm of soil will decrease in concentration due to volatilization and sunlight-mediated photolysis (see Section 3.4.4). Environmental factors controlling the rate of loss include climatic conditions, soil characteristics such as porosity, and the chemical form of the source as particle-bound or dissolved in solvent (Freeman and Schroy, 1989; Paustenbach *et al*., 1992). Vertical migration of dioxins and furans in soils, however, is extremely limited, and levels of dioxins and furans in soils at depths greater than approximately one cm may not decline appreciably over time (Paulausky *et al*., 1986). Dioxins and furans in subsurface sediments also are likely to be highly persistent.

Photolysis of dioxins/furans occurs via progressive dechlorination (Friesen *et al*., 1990). This is also true for most highly chlorinated organic contaminants studied to the present time. Estimated half lives of destruction, calculated from laboratory studies and extrapolated to the environment, range from hours to days. The rate of photolysis generally declines as the number of chlorine substituents increases. Products of photolytic dechlorination often include lower chlorinated congeners (e.g., mono- to tetrachlorinated-dibenzodioxins and furans).

3.4.2 Biodegradation

The ease with which substances in pulp mill effluent undergo microbial degradation is obviously related to the nature of secondary treatment prior to discharge. Essentially all North American pulp mills presently employ primary clarification (settling) and secondary treatment of effluent, using a combination of activated sludge (aerobic) and/or anaerobic systems. Such treatment substantially decreases the biological oxygen demand (BOD) of the final effluent. Some mills have also had success in reducing AOX through biotreatment, which tends to decrease the levels of various effluent constituents through degradation, volatilization and sorption to solids and settling.

Chlorate is a pulp bleaching byproduct specifically associated with use of ClO_2, and if released to some marine aquatic environments may cause adverse effects to some marine brown algae (phaeophytes), especially rockweed (*Fucus* spp.). Chlorate in pulp mill treated effluent, however, occurs at concentrations much lower than those associated with biological effects in the receiving environment (Solomon *et al*., 1993), and the associated ecological risk, therefore, is negligible. Malmqvist and Welander (1994) and others have demonstrated that chlorate can be effectively removed from pulp mill effluent through microbial reduction to chloride. In bench-scale tests, it has been shown that anaerobic bioreactors can achieve 90 to 100% chlorate reduction with hydraulic retention times of approximately 20 to 60 minutes. Slightly lower reduction efficiencies were realized in pilot-scale flow-through bioreactors; i.e., equivalent removal efficiencies of > 80 to 90% required a hydraulic retention time of 1.6 h or greater. Malmqvist *et al*. (1994) isolated a new genus and species of bacterium capable of using chlorate under anaerobic conditions as a terminal electron receptor.

Solomon *et al*. (1993) summarized the treatability of various effluent substances. Efficiencies of removal for chlorophenols, for example, are typically from 50 to 90% following secondary treatment. Larger, hydrophobic organic compounds are predominantly associated with living and detrital biomass, and may be subsequently removed from the effluent stream through sedimentation in treatment lagoons and settling ponds.

As discussed extensively in Solomon *et al*. (1993), secondary treatment of ECF effluent greatly reduces the concentrations of most substances. Yin *et al*. (1994) demonstrated that chlorophenols, chlorocatechols and chloroguaiacols in effluent from two U.S. mills declined in concentration with increasing ClO_2 substitution: 3,4,6-trichlorocatechol and other chlorinated phenolics with three or more chlorine atoms were not detected in untreated ECF effluent. The subsequent secondary treatment in a bench-scale aerated lagoon for six days reduced levels of all analytes in the 100% ClO_2 effluents to 2 mg/L or less; roughly 80% of the analytes were undetected in the biotreated effluent.

Whereas secondary treatment produced a further reduction in the concentrations of lower chlorinated phenolic compounds of 95-100%, AOX removal via biodegradation was only 65% for effluent from 100% ClO_2 substitution. The toxicological significance of the efficiency of biodegradation of the chlorophenolic compounds can be assessed; however, the environmental benefit attributable to AOX removal efficiencies remains unclear. The greater biodegradation of AOX with increased ClO_2 substitution in the study by Yin *et al.* (1994) is possibly due to the production of more highly oxidized and less chlorinated lignin residuals, but the range of structures of the predominantly high MW substances has yet to be adequately resolved.

Lower chlorinated phenols, catechols, guaiacols, vanillins and syringols (< 3 chlorines) in ECF effluents are readily degraded by aerobic biological treatment, with removal efficiencies varying from 29% to 100% (Graves *et al*., 1995). It has been hypothesized that the environmental degradation of high MW effluent materials could give rise to the further depolymerization of lignin constituents rich in polyphenolics, and - in particular - produce potentially toxic monomeric aromatic molecules such as chlorinated phenols. Studies of high MW fractions from kraft mills employing 100% ClO_2 substitution, however, show that such fractions have a low phenolic content, and are primarily non-chlorinated, with a minor monochlorinated portion (tri- and higher chlorinated phenolics have not been detected) (Axegård *et al*., 1993). Degradation of high-molecular weight material from ECF-bleaching in receiving waters should not, therefore, lead to the formation of highly chlorinated monomeric phenolic compounds.

The plant sterols β-sitosterol, campesterol, and stigmastanol exhibited removal efficiencies in secondary treatment systems (aerobic stabilization or activated sludge treatment) of nine U.S. pulp mills in the range of 2 to > 95% (Cook *et al*., 1997). Stigmasterol, a common metabolite of sitosterol biotransformation, generally increased in concentration across the treatment systems; i.e., was substantially higher in the final effluent than in the influent waste. This was undoubtedly due to microbial biotransformation of other sterols or precursors.

Microbial degradation of organic compounds either in the environment or in treatment systems may involve complete breakdown to CO_2, H_2O and other simple molecules (e.g., for carbohydrates and readily degraded organic substances), partial degradation through selective cleavage of carbon-carbon bonds, loss or exchange of functional groups, or — for organochlorines — dechlorination reactions. Zheng and Allen (1996) examined the microbial dechlorination of three monomeric and one dimeric organochlorine that were previously produced during laboratory simulations of sequential ClO_2 and NaOH reactions with 4-methylguaiacol. The model compounds were 4-methyl-5-chloromuconolactone monomethyl ester (MCME), 2-chloro-3-methylmuconlactone (CMML), 3-chloro-4-methylcatechol (CMCA), and 2- (4'-methyl-2'-muconylmethyl)-3-chloro-4-methylmuconic acid dilactone (CMDL). Cultures of a mixed microbial inoculum were able to biologically dechlorinate CMML, whereas MCME, CMCA, and CMDL were not dechlorinated by the aerobic microbial consortia over a 35 day period. The difference in the tendency of different chloromuconic acids or muconolactones to undergo microbial dechlorination was postulated to be caused by differences in the position of a methyl group, where the presence of a methyl group in the -4, but not -3 position may interfere with ortho-cleavage. The study provides a necessary first step developing a mechanistic understanding of the potential for microbial dechlorination of constituents in effluent, as well as molecular structures that inhibit biotreatability.

Several recent studies have examined changes in whole effluent toxicity or group parameters following secondary treatment, rather than examining quantitative changes in individual substituents or fractions. Cates *et al*. (1995), for example, conducted bench-scale simulations of oxygen-delignified hardwood and softwood kraft bleaching with ECF and TCF sequences. Aromatic compounds produced at various stages and estimated as the absorbance at 280 nm were assessed for biodegradability over a six day period by the white-rot fungus *Trametes versicolor*. For all simulations, total absorbances at 280 nm were reduced by 19% to 32%. Aromatics from softwood effluent were more readily degraded than in hardwood effluent. For all treatments, reductions in aromatic compounds by the white-rot fungus occurred primarily in the high MW fraction (MW > 1000 Daltons), an observation that is consistent with the known metabolic abilities of white-rot fungi in their natural environment. A six day treatment of the simulated whole effluent from either ECF or TCF sequences rendered effluents that were non-toxic as measured by Microtox^{TM} bioassays.

Martel and Kovacs (1996) demonstrated that secondary treatment of effluents from thirteen mills (including seven bleached kraft mills) significantly reduced the potential to induce mixed function oxygenase enzymes (i.e., EROD) in laboratory-held rainbow trout. There was no statistically-significant EROD induction in fish exposed to 10% secondary-treated effluents for four of the seven bleached kraft mills. The underlying cause of variations in effluent treatability between mills could not be determined due to the study design. While Martel and Kovacs did not distinguish between ECF and other mills, the important point is that effective secondary treatment has the potential to reduce any risk from most mills, including ECF mills.

3.4.3 Metabolic modification and excretion

Metabolism of specific organochlorines and various non-chlorinated aromatic compounds in fish, birds, mammals and some invertebrates and bacteria has been studied, and metabolism is recognized to be a significant pathway of elimination from the body in at least some organisms. Metabolic modification of aromatic chlorinated organic compounds in higher animals, including all vertebrates, does not involve reductive dechlorination (and destruction); rather, it involves the enzyme-mediated addition of substituents on the aromatic rings such as methylsulfone- or dihydrodiol-groups to carbon atoms that lack a chlorine. Methylsulfone-, dihydrodiol- or dihydroxy-substituted organochlorines are more polar than their non-metabolized precursors. Metabolism, therefore, facilitates elimination (excretion) from the body by increasing the solubility of the molecule in body fluids, and lowering the K_{OW}. For specific classes of compounds, the positions of the chlorine atoms around the aromatic ring structure are the primary determinants of the ease of enzymatic attack and metabolic modification. For example, metabolic modification does not mineralize dioxins and furans or most phenolics (i.e., convert them to CO_2, H_2O and Cl^-), but rather modifies the congener composition through the differential excretion of specific congeners.

3.4.4 Sedimentation and burial

The only other major mechanism of long-term removal of dioxins, furans, and other persistent contaminants from the environment is deposition in stable geological sinks. The most obvious example of this is deep burial in sediments. Immobilization of dioxins/furans in lake and marine sediments is enhanced in areas of rapid sedimentation, such as at the ends of river deltas and in deep catchment basins. The reworking of sediments by burrowing infauna, or resuspension due to turbulence associated with tides and storms have the potential to remobilize organochlorines from sediments. Extended residence time of contaminants in the water column prior to and during settling would delay the removal to sediment deposits, as well as enhance bioavailability to free swimming (pelagic) organisms.

The extent to which historically deposited dioxins, furans and other effluent derived substances continue to exert environmental impacts is determined in part by the potential for remobilization from sediments to biota and/or the overlying water column. Remobilization from sediments in settling ponds can also be a problem. For example, the discharge of final effluent from a British Columbia mill contained up to 200 parts per trillion 2,3,7,8-TCDF after process changes up to 1993 virtually eliminated this contaminant from effluent entering the settling pond (Pagoria and Kerfoot, 1996). Elevated furans in the final effluent were attributed to resuspension and entrainment of previously contaminated aerated lagoon sediments. Similarly, there were fourteen documented exceedences of regulatory limits for 2,3,7,8-TCDF or -TCDD (50 ng/L and 15 µg/L respectively) in 1994 at five British Columbia pulp mills (Krahn, 1995). Most of the exceedences were attributable to re-suspension from treatment lagoon sludges and combustion of salt water laden hog fuels.

Losses of the model dioxins 1,3,6,8-TCDD, 1,3,7,9-TCDD, HpCDD and OCDD from surface sediments in freshwater mesocosms (littoral enclosures) were attributed primarily to remobilization to the water column via resuspension and upward diffusion of DOC-associated dioxins (Segestro *et al*., 1996). Estimated half lives of loss ranged from 4.4 to 6.2 years, and sediment- sorbed dioxins remained bioavailable to freshwater mussels and crayfish five years after the original introduction of dioxin-contaminated sediments.

Hagen and Colodey (1995) provided an overview of the responses between 1989 and 1993 of dioxin/furan bioaccumulation in Dungeness crabs (*Cancer magister*) in coastal areas of British Columbia near pulp mill discharges. Dioxin and furan concentrations in Dungeness crab hepatopancreas have generally declined substantially; however, some areas have undergone a slower recovery than others. There are a number of possible explanations for the observed variations in recovery rates. Local differences in sedimentation rates are probably a major factor.

In general, the presently available studies on biodegradation and removal processes for constituents of pulp mill effluent emphasize the importance of effective secondary treatment in the reduction or elimination of whole effluent toxicity. Subsequent to past improvements in kraft mill effluent quality realized through ClO_2 substitution, future reductions in toxicant inputs where they are still occurring might be achieved *through improvements in treatment, since (i) recent studies suggest that many bioactive compounds in final effluent are non-chlorinated compounds contributed by pulp mill processes other than bleaching, and (ii) most of the known deleterious substances in pulp mill effluent are amenable to biodegradation and/or removal through adsorption to particulates. With regard to dioxin and furan levels historically released to aquatic environments, the primary mechanism for ecosystem recovery is likely to be through burial in subsurface sediments where there is little potential for sediment resuspension or interaction with aquatic fauna.*

3.5 TEMPORAL TRENDS IN DIOXINS/FURANS IN THE RECEIVING ENVIRONMENT

A large number of studies have documented profound decreases in the concentrations of potentially deleterious substances in final effluent, water, sediment or aquatic organisms after curtailment of elemental chlorine for bleaching pulp. For example, Owens *et al*.(1994) observed decreases in the levels of most chlorophenolic substances in effluent from an Alberta pulp mill, and in the water column to near the analytical detection limits following conversion to 100% ClO_2 substitution. Complete ClO_2 substitution resulted in the virtual elimination of polychlorinated phenolics in final effluent.

Abbott and Hinton (1996) recently summarized monitoring data for the aquatic receiving environment of 39 mills in the US. Overall, the data indicate a median rate of decline in the concentrations of 2,3,7,8-TCDD in fish tissues (whole fish and fillets: lipid-normalized concentrations) of 36% decrease per year since 1989. Similar reductions in tissue concentrations occurred for pelagic and benthic species. An overall decline in levels of 2,3,7,8-TCDD in the effluent from 104 U.S. pulp mills between 1988 and 1993 occurred at a median rate of 18% per year. The benefit associated with declines since the late 1980s in dioxins and furans in fish tissues is perhaps best illustrated by the diminishing number of dioxin consumption advisories in the U.S.; i.e., the lifting of dioxin advisories in 17 of 30 waterbodies downstream from pulp mills where advisories were in effect at the end of 1990 (AET, 1995).

Krahn (1995) summarized trends in the effluent concentrations and environmental loadings between 1987 and 1994 of 2,3,7,8-TCDD and TCDF for twenty six pulp mills in British Columbia, Canada, including unbleached kraft mills. The temporal trend in loadings is illustrated in **Figure 10**; temporal trends in final effluent concentrations of 2,3,7,8-TCDD and 2,3,7,8-TCDF are summarized in **Figure 11**. Declines, commencing around 1989, are primarily attributable to substitution of ClO_2 for Cl_2 in bleached kraft mills, and also likely due to better scrutiny of process control; e.g., leading to less black liquor carry over into the bleaching process. Other studies documenting substantial declines in dioxins/furans in the receiving environment of BC pulp mills include Derksen (1995), Sekala *et al*. (1995), Mellor *et al*. (1995), Beatty-Spence and Antcliffe (1995), Hagen and Colodey (1995) and van Oostdam (1995).

Studies undertaken as part of the first round of the Environmental Effects Monitoring (EEM) Program in Canada clearly show dramatic overall decreases in dioxin and furan concentrations in

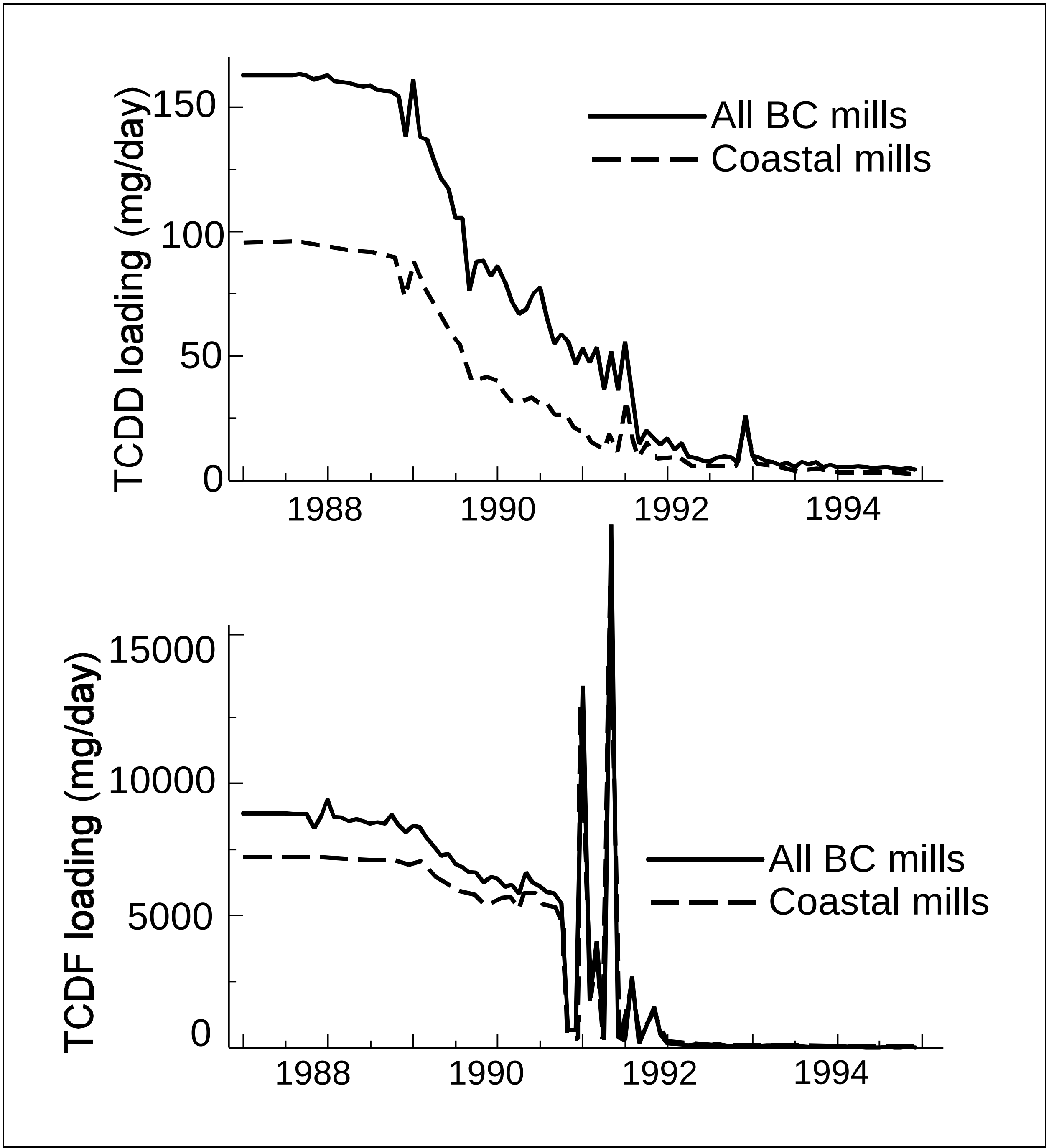


Figure 10 Temporal trends in total TCDD/TCDF loads from pulp mills in BC, Canada.

final effluent, and commensurate improvements in concentrations in various environmental compartments. Few Canadian mills exhibited detectable concentrations of 2,3,7,8-substituted dioxin or furan congeners in final effluents during 1995 (see also Carey *et al*., 1996).

In coastal pulp mills in British Columbia, process changes since 1987 have, in most cases, resulted in substantial decreases in the overall concentrations of chlorinated dioxins and furans in hepatopancreatic tissue of Dungeness crabs, *Cancer magister*, collected near bleached kraft pulp mills, as well as decreases in the portion of dioxins and furans comprised of the more toxic 2,3,7,8-substituted congeners (Yunker and Cretney, 1995). The tetrachlorodibenzofurans have declined more rapidly than the hexachlorodibenzo-p- dioxins. Calculated 2,3,7,8-TCDD TEQs in 1987 for crab hepatopancreas samples from all coastal mills fell in the range 82 to 2,900 pg/g wet weight; as of 1992 to 1994, the range of values was 7.2 to 99 pg/g wet weight. Additional crab data from 1995 to the present time further support a synoptic decrease in dioxin and furan concentrations in the receiving environment; however, the more recent data have not yet been collated (Yunker and Cretney, personal comunication).

The relatively rapid decline in tissue burdens in Dungeness crabs at some coastal mills is initially surprising, given historical deposits of dioxins in sediments up to the early 1990s, the known environmental persistence of 2,3,78-TCDD and other more highly chlorinated congeners, and tendency of crabs to interact with shallow subsurface sediments through foraging and shallow burrowing activities. It is likely that differences in ecosystem recovery rates at different BC coastal mills are related to local differences in sedimentation rates and/or scouring rates: Aquatic receiving waters of mill effluent near substantial riverine input and, hence, higher rates of sedimentation, or in fjords that serve as large- scale sediment traps, appear to be undergoing a more rapid recovery from historical dioxin and furan inputs.

Recent temporal trends in environmental concentrations of 2,3,7,8- substituted dioxins and furans clearly show marked decreases in inputs from ECF mills, with virtually all North American mills having already achieved virtual elimination of 2,3,7,8-TCDD and the vast majority having achieved elimination of 2,3,7,8-TCDF. Corresponding but incomplete recoveries in tissue concentrations in fish and shellfish populations have also been documented.

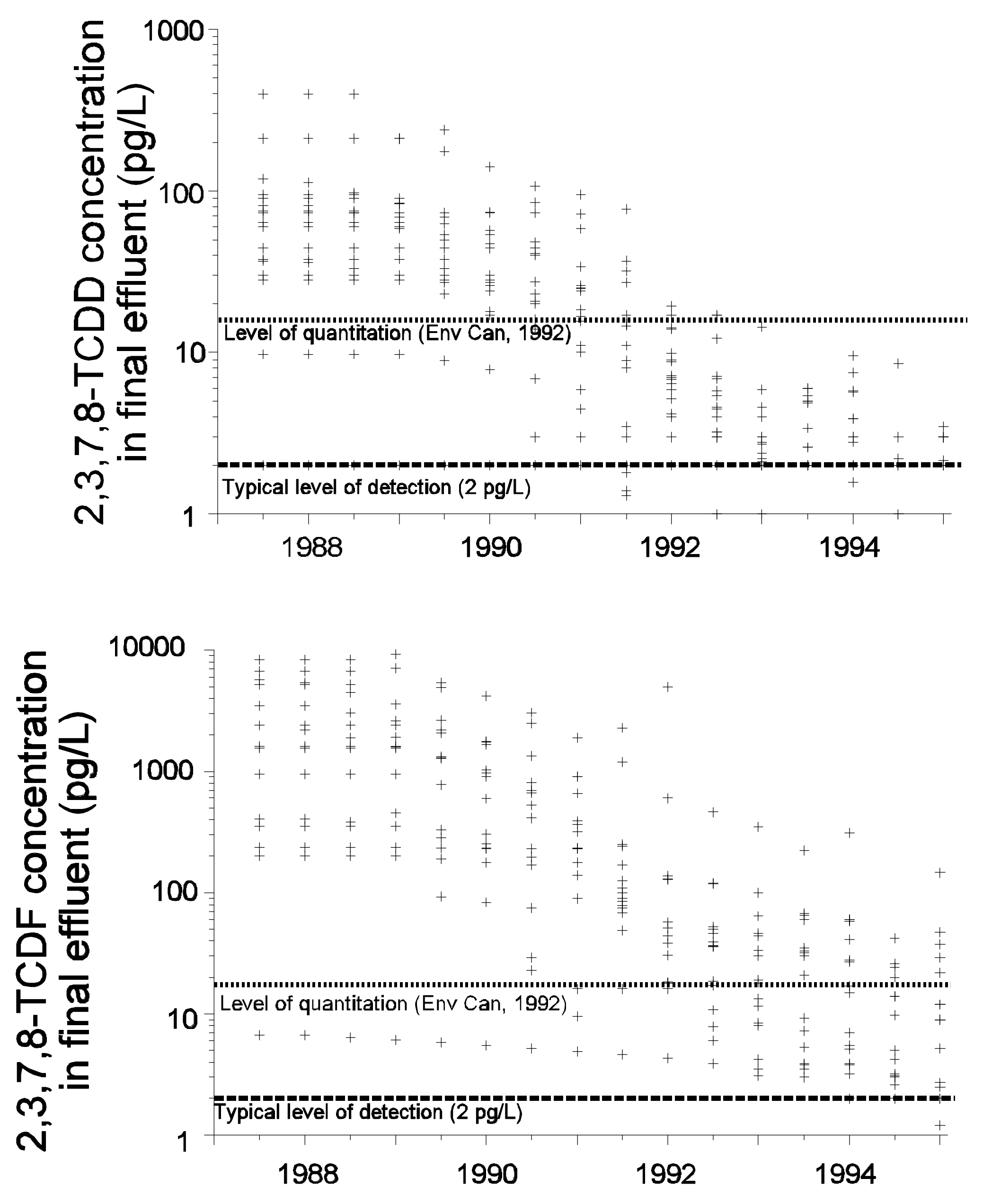


Figure 11 Temporal trends in concentrations of TCDD/TCDF reported in mill effluents from BC, Canada.

4 EFFECTS CHARACTERIZATION

The previous review of the ecological risks associated with chlorine dioxide bleaching (Solomon *et al*., 1993) concluded that substitution of chlorine dioxide for chlorine results in a reduction in toxicity of pulp mill effluents and a reduction in adverse ecological effects. The report stated that "mills bleaching with high chlorine dioxide substitution (100%), employing secondary treatment and with receiving water dilutions typical of most mills in North America, present an insignificant risk to the environment from organochlorine compounds". The previous review also identified research needs related to effects including:

Identification of the compounds in biologically treated effluents responsible for MFO induction and plasma steroid hormone reduction;
Assessment of the sublethal toxicity of effluents to the species most severely impacted at the population level;
Understanding the potential effects of chlorate in freshwater environments where nitrogen is limiting and;
Assessment of the extent to which organochlorine compounds in sediment are released from sediments either directly or through benthic food chains, and the extent to which this release confounds the understanding of the relationship between responses of fish and the current quality of effluents.

This chapter begins with a brief summary of previous knowledge of the effects of pulp mill effluents (BKME) on aquatic biota. It describes recent research on the causes of these effects and on the relationship of effects to pulping, bleaching and effluent treatment technologies, particularly ECF vs TCF bleaching. New data were not found that addressed directly the questions of the release of sediment-borne compounds, the effects of chlorate in freshwaters, or the nature of effects on severely affected species.

4.1 EFFECTS OF PULP MILL EFFLUENTS ON FISH

Land-mark field studies in Sweden demonstrated that the discharge of BKME can cause important detrimental effects on fish populations (see review by Hodson *et al*., 1996a). The most obvious effects were caused by older mills, characterized by bleaching with elemental chlorine, poor spill and carbon recovery systems, and little or no effluent treatment. In fact, one of the mills in the study was operating very poorly because it was being up-graded during the study. Fish downstream accumulated chlorinated organic compounds and showed physiological responses indicative of stress and of changes in rates of metabolism of xenobiotic substances, increased excretion rates of compounds found in BKME, increased prevalence of gross deformities, reduced recruitment, and shifts in fish population and community structure. Improvements to mill processes, replacement of chlorine with ClO_2 as a bleaching agent, and secondary effluent treatment sharply reduced the discharge of chlorinated compounds and the contamination of fish. Many of the signs of toxicity, such as gross deformities, were eliminated, but some physiological responses remained, particularly increased rates of metabolism of xenobiotics. The Swedish EPA initially interpreted the reduction in effects as a response to reduced loadings of chlorinated organic compounds. However there were no chemical analyses of non-chlorinated compounds in effluent or fish, (Tana and Lehtinen 1996; Owens 1991), so their contribution to toxicity was unknown.

The effects on fish of BKME were later confirmed in North America, but the newer studies led to a different interpretation — effects were likely due to natural constituents of wood. The North American studies also demonstrated that fish downstream of some bleached kraft mills showed differences in the serum concentrations of reproductive steroid hormones. Coupled with changes in fish population demographics, such as reduced gonad size, reduced fecundity, delayed maturity, and increased size at maturity, the hormone responses were consistent with the idea that population impacts of BKME are due to impaired reproduction.

The North American studies differed from the Swedish studies in that effects on fish were not unique to kraft mills nor to chlorine bleaching. Effects on steroid hormone concentrations and induction of MFO enzymes were also observed in fish downstream of kraft, sulfite, thermomechanical pulping (TMP) and chemi-thermomechanical (CTMP) mills not using bleaching. Increased rates of deformities and skin lesions seen in Sweden were less obvious in North America (Couillard *et al*., 1995). Further, many of the physiological responses attributed to effluents could be accounted for as responses to seasonal variations in fish physiology due to natural gradients of habitat and growth rates (Hodson *et al*., 1992a). Physiological measurements are also biased by capture stress, which cannot be alleviated by storing fish in cages before sampling (McMaster *et al*., 1994; Jardine *et al*., 1996a; 1996b). Effects on demographics were sometimes confused by the variable growth response; reproduction enhanced by nutrient-stimulated growth rates may have obscured impairment of reproduction by toxicity (Gagnon *et al*., 1995; 1996).

The importance of secondary treatment was also uncertain. At several mills in Ontario where secondary treatment and increased ClO_2 substitution were initiated, effects on fish in receiving waters were reduced but still evident (Munkittrick *et al*., 1992). More importantly, studies of some bleached kraft mills in Western Canada found few effects (Swanson *et al.*, . 1994; Kloepper-Sams *et al.*, 1994), indicating that effluent toxicity was not universal. The technological basis for differences in effects are not at all clear, but there are an increasing number of studies demonstrating the benefits of both ClO_2 substitution and enhanced effluent treatment, in terms of reduced chemical contamination and reduced physiological responses of fish (e.g., Oikari and Holmbom, 1996).

Field studies can demonstrate which technologies are not uniquely responsible for observed effects on fish; bleaching with chlorine or chlorine dioxide is not a necessary process for pulp mill effluent to have effects on fish. The nature of field studies, in which different observations can only be correlated, and in which numerous factors act simultaneously and independently, prevents the development of clear cause-effect relationships. Early conclusions ascribing BKME effects solely to organochlorine compounds were incorrect.

4.1.1 Physiological basis for effects

This section reviews recent advances in the understanding of the mechanisms, causes and consequences of two frequently-observed responses of fish to pulp mill effluents: changes in metabolism by enzymes and impaired regulation of reproductive steroid hormones. Knowledge of mechanisms provides a basis for assessing whether chlorinated compounds play a role in these responses, and whether ECF bleaching is an important source.

4.1.1.1 MFO Induction

Mixed-function oxygenases (MFO) are enzymes that oxygenate endogenous and exogenous low molecular weight substrates. Activity of MFO enzymes, such as is measured by ethoxyresorufin-O-deethylase (EROD) activity, increases following exposure of fish to pulp mill effluents. This increase in activity is called induction. Significant MFO induction indicates exposure and potential adverse effects; conversely, the absence of induction is evidence that either effluent does not contain sufficient inducers to elevate MFO activity, or that MFO inhibition has occurred due to high concentrations of other chemicals (e.g., resin acids) (Hodson *et al.*, 1996c).

The potential adverse effects associated with MFO induction may be due to unrelated toxicities of inducing compounds (i.e., induction simply indicates the degree of exposure) or to the oxygenation of compounds by MFO enzymes. Oxygenation of aromatic compounds creates reactive intermediates such as epoxides and alcohols that may be conjugated to amino acids. These conjugates are more polar than the parent compound, and are readily excreted in the bile. Hence, increased activity can reduce toxicity by reducing exposure. Production of reactive intermediates can also be harmful. Some cause strand breaks in DNA or react readily with nucleic acids, forming “adducts” that can cause mutations, impaired replication of DNA, and sometimes cancer. Adducts have recently been reported for the first time in chinook salmon (*Oncorhynchus tshawytscha*) exposed naturally or in the laboratory to BKME (ECF, 8-d aerated stabilization pond) from a mill at Prince George, B.C. (Wilson, 1997). An elevated frequency of micronuclei in erythrocytes of chinook salmon exposed for 30 d to effluent from the same mill (Easton *et al.,* 1997) is also consistent with a mutagenic response. The effect was observed at 4% (v/v) effluent or greater. Another consequence of reactive intermediates is the oxidation of other substrates, such as polyunsaturated lipids. Where exposures are low, the anti-oxidant defense system may neutralize the reactive intermediates; this system includes enzymes that catalyze reducing reactions, reductants such as glutathione, and antioxidant vitamins, such as vitamin C and E. If there is sufficient oxidation, a condition of oxidative stress may occur, in which lipids begin auto-peroxidation (rancidity), leading to cell membrane damage and cell death.

Prolonged MFO induction by TCDD, and hence prolonged activation of the *cypIA1* gene has been associated with a suite of toxic effects known as receptor mediated toxicity (RMT), which includes involution of the thymus, wasting, and cell membrane damage (Okey *et al.,* 1994). In fish the effects are most evident in larvae, which exhibit “blue-sac” disease, characterized by edema (particularly of the yolk sac), cranio-facial deformities, fin erosion, and hemorrhaging, particularly around the heart. The condition is fatal (Spitsbergen *et al.,* 1991). RMT can be caused by other persistent inducers such as co-planar PCBs, chlorinated dioxins, and chlorinated furans, and potency for blue sac disease is similar to potency for MFO induction (Walker and Peterson 1991; Parrott *et al.*, 1995a). Prolonged exposure to retene (methyl-isopropylphenanthrene) also causes prolonged MFO induction and symptoms of blue sac disease in developing trout (N. Fragoso, S. Billiard, and P. Hodson, unpublished data).

Despite an association of MFO induction with different mechanisms of toxicity, the significance of induction in wild fish is difficult to interpret. The duration of chemical exposure and MFO induction is usually unknown and the extent of induction associated with toxicity has not been characterized. Further, toxicity is usually assessed with laboratory tests of juvenile stages of trout, while induction in field studies is measured in adults of other species. For example, high concentrations of dioxins were found in whitefish near a mill in Saskatchewan, without apparent adverse effects (Kloepper-Sams and Benton 1994).

4.1.1.2 Regulation of reproductive steroid hormones

The disruption of reproductive steroid hormone regulation in fish (Munkittrick *et al.*, 1991) provides a plausible mechanism for observed reproductive impairment, as shown by delays in age to maturity, smaller gonad size, and reduced fecundity. Research since 1993 has indicated that one of the candidate compounds for causing these effects is β-sitosterol, a non-chlorinated natural constituent of wood, and the most abundant plant sterol released during pulping (Stuttaford, 1995). Sitosterol can be metabolized by soil bacteria to a variety of androgenic and estrogenic compounds, and this process has been shown to cause changes in secondary sexual characteristics of tropical fish species (e.g., *Heterandria* sp., Davis and Bartone, 1992). Debarking effluent, abietic acid, and sitosterol have been found to cause vitellogenesis in fish, a sign of an estrogenic response (Mellanen *et al.*, 1996), and injected sitosterol interferes with reproductive steroid hormone regulation of goldfish (*Carassius auratus*; MacLatchy and Van Der Kraak, 1995). While injection of goldfish with sitosterol causes a change in serum concentrations of reproductive steroid hormones, it is not clear how sitosterol is acting and whether concentrations in final effluent are sufficient to cause the observed reproductive impacts on fish. Very little sitosterol seems to survive past secondary treatment (<10 µg/L; Stuttaford, 1995; also see section 3.4.2). Further, while endocrine disruption by pulp mill effluent exposure was found consistently at some mills, the point in the synthetic pathway of reproductive steroid hormones where pulp mill effluent exposure causes impairment is still not well defined (McMaster *et al.*, 1995).

A review by McMaster *et al*. (1996a) of a series of field studies indicated that reproductive impairment, as shown by changes in hormone concentrations or rates of hormone synthesis, is frequently found in fish downstream of pulp mills. However, the pattern of response is not universal, because fish exposed to BKME in the Androscoggin River in Maine showed elevated serum testosterone concentrations, in contrast to fish sampled at other BKME-contaminated sites (McMaster *et al*., 1996a). Some species such as brown bullhead (*Ictalurus nebulosus*) were also less sensitive compared to white sucker and steroid effects could also be found in fish exposed to polynuclear aromatic hydrocarbons (PAH) from steel mills. Understanding the relationship of these responses to pulp mill technology will require an understanding of the biochemical basis of the effect and of the compounds causing those effects. However, it was obvious that reproductive effects could occur in the absence of ECF bleaching, as shown by reproductive responses of fish from the Winnipeg River, sampled downstream of a kraft mill not using chlorine compounds for bleaching.

No links have yet been demonstrated between MFO induction and impairment of reproductive responses (e.g., Swanson *et al.,* 1994); they may have entirely separate causes and consequences. While some compounds causing induction also cause reproductive impairment (e.g., co-planar PCBs, TCDD, PAH; McMaster *et al*., 1996a), the mechanisms may be independent. In fact, increased estrogen in female fish during the final stages of sexual maturation “down-regulates” the *cyp450* gene so that MFO induction is reduced.

Zacharewski *et al*. (1996) described a transfected yeast bioassay that reacts to estrogen-like compounds; this “reporter-gene” bioassay was developed as a screening tool. They tested undiluted black liquor from a bleached kraft pulp mill as well as a specific fraction isolated from the secondary-treated effluent from the same mill. In previous tests, both samples caused MFO induction in fish and, in this yeast bioassay, both samples demonstrated estrogen-like activity. However, it is unknown at this time whether the two responses involve the same chemicals.

The relationships between MFO induction and effects on fish of pulp mill effluent are as yet undefined. MFO induction and alterations in serum concentrations of reproductive steroid hormones appear to be separate and unrelated responses of fish to pulp mill effluents. While the hormone response may be the mechanism by which pulp mill effluents affect fish reproduction, the specific details are not completely worked out. The toxicological significance of MFO induction is related to prolonged elevation of MFO activity and prolonged gene activation. Both steroid effects and prolonged MFO induction can be caused by non-chlorinated natural constituents of wood produced by pulping, not by ECF bleaching.

4.1.1.3 Causes of MFO induction

There have been numerous studies demonstrating MFO induction in fish downstream of pulp mills, and exposed to effluents, diets, sludges, and sediments in the laboratory (Table 4-1). The consistency of the response and its possible connection to toxicity (see above) has prompted a search for inducing compounds and for pulp mill processes controlling their concentrations in effluent. The search first focused on chlorinated dioxins and furans because of their high potency for induction (Parrott *et al*., 1994), their occurrence in effluent from mills using chlorine bleaching (Berry *et al*., 1991), and the close relationship between dioxin equivalents in fish tissues and measures of MFO induction (Hodson *et al*., 1992b; Kloepper-Sams and Benton 1994). At mills where elemental chlorine was replaced by ClO_2, dioxin and furan emissions were substantially eliminated, concentrations in sediments and biota decreased dramatically, and fisheries were reopened (see Sections 2.3.2; and 3.5). However, MFO induction in fish from receiving waters did not disappear at all locations (Munkittrick *et al*., 1992; van den Heuvel *et al.*, 1996), and induction was found in fish exposed to effluents from all types of mills, including those not using chlorine or ECF bleaching (Munkittrick *et al*., 1994; Gagné and Blaise, 1993; Martel *et al*., 1994). These results were confirmed in a more controlled experiment in which bass (*Micropterus salmoides*) and catfish (*Ictalurus punctatus*) were exposed to BKME in experimental streams for periods of up to 263 d (Bankey *et al.*, 1995). Fish exposed to 4-8% (v/v) effluent from a mill using 70% ClO_2 substitution were induced 55-fold over controls, but induction was lost rapidly when the fish were transferred to fresh water. TCDD concentrations in effluent ranged from <6 - 20 pg/L, while TCDF concentrations were about four times greater. A subsequent exposure of fish to 4-12% (v/v) effluent after the mill switched to oxygen delignification and 100% ClO_2 substitution also demonstrated MFO induction, but in this case, the potency was much less and induction was only 3-fold over controls. For the ECF effluent, TCDD concentrations were lower but exceeded the detection limit of 5 pg/L in 5% of samples; TCDF was detectable in 47% of samples. Hence, while dioxins and furans may have contributed to early measurements of induction, they were not the only cause, and they are certainly not the current cause.

Hewitt *et al*. (1996) concluded that resin acids, chlorophenols, aliphatic alkanes, plant sterols, chlorinated dimethyl sulfones and terpenes were not the cause of MFO induction in trout exposed to fractions of BKME; these compounds were found at high concentrations in effluent fractions that did not cause induction. Fish and cell culture bioassays of MFO induction by compounds tested singly confirm that chlorophenols, resin acids, and sitosterol are not inducers (Lehtinen, 1996; Hodson and Parrott, unpublished data), but defoamers cause slight induction at very high concentrations (Hodson and Parrott, unpublished data).

Pulping liquors, both weak black liquor from kraft pulping and spent liquor from alcohol pulping, contain high concentrations of MFO inducers, as shown by bioassay (Hodson *et al*., 1997). Small losses of black liquor to effluent could explain the induction potency of final effluent. Bleaching effluents may also be a large source of inducers, but secondary treatment systems can be an efficient means of reducing the MFO induction potential of effluents (Schnell *et al*., 1993; Martel and Kovacs, 1996a).

Inducers can be extracted from wood chips using dichloromethane, with about the same efficiency of removal as with alcohol pulping (Hodson *et al*., 1997), indicating that inducers are natural substances extracted from wood. The results are consistent with earlier studies of MFO induction in rainbow trout liver cells exposed to extracts of bleached and unbleached kraft and unbleached sulphite mill effluents, and in perch downstream of bleached and unbleached mills (Hanninen *et al*., 1991; Pesonen and Andersson 1992).

Table 4-1 Compounds, effluents, and process streams from pulp mills that are associated with specific physiological responses of fish. Blank cells indicate no information or “not applicable”.

Source	Test System	Possible Agent	Mill Type	Bleaching sequence	Effluent treatment^a	Reference
MFO induction						
Whole effluent	White sucker and pike, receiving water	chlorinated dioxins and furans	Bleached Kraft	Chlorine		Hodson *et al.*, 1992b
Whole effluent	Longnose sucker and whitefish, receiving water	chlorinated dioxins and furans	Bleached kraft	25-70% substitution	Clarifier 5 d ASB	Kloepper-Sams and Benton 1994
Whole effluent	White sucker, receiving water	***Not*** chlorinated dioxins and furans	Bleached and unbleached kraft, CTMP	Chlorine, ECF, unbleached		Munkittrick *et al.*, 1994; Williams 1993
Whole Effluent	Bass and catfish, experimental streams	***Not*** exclusively chlorinated dioxins and furans	Bleached kraft Bleached kraft	70% substitution Oxygen delignification, 100% substitution		Bankey *et al.*, 1995
Whole effluent	Trout, lab	***Not*** exclusively chlorinated compounds	Bleached kraft Bleached kraft Bleached kraft CTMP TMP	ECF, oxygen delignification ECF, no delignification Partial substitution	primary	Martel and Kovacs 1996b
Whole effluent	Trout, lab	***Not*** AOX, chlorophenols, resin and fatty acids	Bleached kraft Bleached kraft Bleached kraft Bleached kraft Kraft	chlorine 30-60% substitution 50 % substitution ECF None	primary secondary secondary secondary secondary	Williams 1993
Pulping liquors; wood	Trout, lab	extractives				Hodson *et al.*, 1997

Source	Test System	Possible Agent	Mill Type	Bleaching sequence	Effluent treatment[a]	Reference
Pulping liquor, whole effluent (balsam fir)	Trout, lab	juvabione, dehydrojuvabione				Martel *et al.*, 1996C
Effluent streams	Trout, lab		Black liquor Oxygen Delignification Chlorine bleachery Extraction Hypochlorite Chlorine dioxide Untreated whole Treated whole	30-60% substitution	Secondary	Schnell *et al.*, 1993
Extracts of treated effluent	Trout hepatocyte culture		BKME (N=3) KME Sulphite	16-50% substitution Unbleached Unbleached	Secondary	Pesonen and Anderson 1992
Whole effluent	Trout hepatocyte culture Caged perch, receiving water		bleached kraft unbleached sulphite	Partial substitution	Clarifier, 2-3 d ASB AS	Hanninen *et al.*, 1991
Sludges (in diet)	Trout, lab		Bleached kraft			Lehtinen *et al.*, 1991
Sludges, downstream sediments	Trout, lab		Bleached sulphite			Parrott and Hodson, unpublished data
Fractions of whole treated effluent	Trout, lab	***Not*** resin acids, chlorophenols, aliphatic alkanes plant sterols chlorinated dimethyl sulfones, terpenes	Bleached kraft	50% substitution	Clarifier 7 d ASB	Hewitt *et al.*, 1996
Fractions of whole treated effluent	Trout, lab	alkyl-substituted PAH; chlorinated stilbene; chlorinated diarylethane	Bleached kraft	50% substitution	Clarifier 7 or 10 d ASB	Hodson 1996; Burnison *et al.*, 1996; 1997

Source	**Test System**	**Possible Agent**	**Mill Type**	**Bleaching sequence**	**Effluent treatment**[a]	**Reference**
Proprietary Mixture	Trout, lab	Oil-based defoamers				Hodson and Parrott, unpublished data
Single compounds	Trout, lab	***Not*** chlorophenols, resin acids, ß-sitosterol				Lehtinen *et al.*, 1996; Hodson and Parrott, unpublished data
Single compounds	Trout, lab	Chlorinated dioxins and furans				Parrott *et al.*, 1995a
Single compounds	Trout, lab	retene				Parrott *et al.*, 1995b
Single compounds	Trout, lab	juvabione, dehydrojuvabione				Martel *et al.*, 1996c
Endocrine disruption						
Debarking effluent	Trout, lab	ß-sitosterol abietic acid				Mellanen *et al.*, 1996
Whole effluent	Wild white sucker *In vitro* White sucker ovarian follicles		Bleached kraft			McMaster *et al.*, 1995
Whole effluent	goldfish, *In vitro* ovarian follicles		Kraft, bleached and un-bleached			McMaster *et al.*, 1996b
Black liquor	cell culture		Kraft			Zacharewski *et al.*, 1996
	goldfish, *In vitro* ovarian follicles	ß-sitosterol				MacLatchey and Van Der Kraak 1995

[a] ASB - Aerated Stabilization Basin; AS - Activated Sludge

Burnison *et al*. (1996) isolated MFO-inducing fractions of secondary-treated effluent from a mill using 50% ClO_2 substitution. Bioassays with rainbow trout demonstrated that inducers were present in the aqueous phase and associated with dissolved organic matter and particulates (the fractions containing the largest amount). Inducers could be extracted from particulates by methanol and from the aqueous phase by adsorption to C-18 resins. Extracts cleaned up on C-18 resin were separated by reverse-phase HPLC using methanol, and induction was isolated primarily to one fraction, along with standard marker substances with log K_{OW}s of 4.6 - 5.1. Therefore the inducer appeared to be moderately hydrophobic, slightly soluble in water, and easily accumulated from water by fish. Compounds identified by preliminary scans with GC/MS included both PAHs (alkyl-substituted phenanthrene) and chlorine-substituted diarylethanes and diaryl ethenes (stilbenes) (Hodson, 1996). More recently, Burnison *et al*. (1997) reported that tri-chlorinated stilbenes were the most common constituent in HPLC fractions tested by MFO bioassays with cell cultures, although the identity of this compound must still be confirmed. In this case, 67 fractions were separated by HPLC, and activity was isolated to one fraction only. Engwall *et al.* (1997) have shown that polyaromatic substances extracted from bottom sediments in pulp mill effluent receiving waters contain high concentrations of MFO inducers that were chemically different from the dioxins and furans and the 15 priority PAH pollutants.

The terpenes, juvabione and dehydrojuvabione have recently been identified as inducers that were isolated from balsam fir (Martel *et al*., 1996c). In effluents from mills pulping balsam fir, the extent of induction was accounted for entirely by the concentrations of juvabione present. Juvabione is not found in other wood species commonly pulped in Canada, but it illustrates that a variety of natural wood constituents may be inducers. Other terpenes have been reported to not induce MFO activity (Hewitt *et al*., 1996).

Retene is an inducing compound found in pulp mill effluents, sludges from pulp mill treatment ponds, and in sediment downstream of pulp mills (Judd *et al*., 1995); its origin is thought to be microbial biotransformation of abietic and dehydroabietic acid under anaerobic conditions (see Sections 2.4 and 3.3). Bioassays have demonstrated that retene and other alkyl-substituted phenanthrenes cause 10 to 100-fold MFO induction in rainbow trout (Parrott *et al*., 1995b). Induction can be sustained during prolonged exposure to retene, but declines rapidly when fish are transferred to clean water (Fragoso *et al*., 1996), likely due to retene metabolism and excretion; it is measurable in high concentrations in the bile of fish following exposure (Stuthridge *et al*., 1995). In fact, all of the wood extractives associated with biological responses have structures that appear to be susceptible to metabolic attack, conjugation and excretion.

MFO induction in fish is a common response to many pulp mill effluents, and not a characteristic unique to bleaching. MFO-inducing compounds in biologically-treated effluent are natural extractives of wood, their metabolic by-products, or their chlorinated derivatives. These inducing compounds appear to be non-persistent and easily metabolized and excreted by fish. The relative amounts of these compounds released by ECF or TCF effluents are unknown.

4.2 RELATIONSHIP OF EFFECTS TO PULPING, BLEACHING AND EFFLUENT TREATMENT TECHNOLOGY.

Numerous comparisons of effects on fish have been made among different pulping, bleaching and effluent treatment technologies. However, as was the case with Project Environment Cellulose I and II, studies conducted since 1993 have not clearly related specific pulp mill technology to specific effects.

4.2.1 Field studies

Effluents from 11 Ontario mills were tested for effects using 7-d *Ceriodaphnia* reproduction and survival tests and 7-d fathead minnow (*Pimephales promelas*) survival and growth tests (Robinson *et al.,* 1994; Munkittrick *et al*., 1994; Servos *et al*., 1994; Van den Heuvel *et al*., 1994). Simultaneously, serum concentrations of reproductive steroid hormones and liver MFO activity were measured in male and female white sucker sampled downstream of the same mills and at reference sites. The results demonstrated that effluents from all types of mills cause potential reproductive effects and MFO induction in adult fish in receiving waters, but that effluents receiving secondary treatment were less toxic according to laboratory toxicity tests. There was insufficient information to really compare effects to pulp mill technologies, although some mills that caused effects (sulfite/mechanical mills) did not use any bleaching. Hence, the effects measured could not be associated with the presence or absence of chlorine. Of the four kraft mills having only primary treatment, one used a traditional bleaching sequence (CEH), and the others ClO_2 substitution ranging from 16-30% to 100% (i.e., ECF). Effluent bioassays demonstrated that the ECF mill caused no effect on fathead minnow survival or growth and no effect on *Ceriodaphnia* survival and reproduction (Robinson *et al*., 1994). Mills using the traditional sequence and 50-60% substitution caused increased mortality rates of fathead minnow larvae; only effluent from the mill with 16-30% substitution inhibited *Ceriodaphnia* reproduction. For these 4 mills, there was no relationship between bleaching sequence and MFO induction, serum reproductive steroid hormone concentrations, and dioxin equivalents in white suckers from receiving waters. Differences in water use, receiving water dilution and process efficiencies prevented further useful analyses.

Reductions in effects on fish have been observed at some BKMs, but the relationship to changes in technology are not obvious. McMaster *et al*. (1996b) reviewed a series of studies of a mill at Jackfish Bay, Ontario, where reproductive steroid hormones were measured in serum of native white sucker exposed *in situ*, and where goldfish were exposed experimentally. Rates of hormone synthesis by isolated gonadal tissue of goldfish were reduced in early studies, but between May, 1993 and May, 1994, the extent of effluent effects on goldfish hormones disappeared, coincident with unspecified changes to mill processes. While these included an increase in ClO_2 substitution from 50 to 70%, the majority of changes were not made public, and effects on white sucker steroid hormones were still detectable in late 1993.

Field studies of pulp mill effluent effects on fish suggest responses of fish to effluent can be found with or without bleaching. Secondary treatment alleviated acute toxicity to fish and invertebrates in laboratory tests, but responses in some fish from *receiving waters are still evident, although reduced. These studies demonstrate no other obvious relationships between pulp mill technologies and biological responses.*

4.2.2 Mesocosm studies

Tana *et al*. (1994) studied brackish water mesocosms treated with four effluents from mills with different bleaching sequences: CEHDED; O[C85D15][EO]DED; 2 mixed streams: (O[C60D40][EO]D) and (O[C60D40][EO]DED); and OPD. The acute lethalities of the 4 effluents were 23, 55, 27 and 100% v/v, respectively, with toxicity emission factors of 530, 70, 130 and 30, respectively. Mesocosms were exposed to effluent diluted either 200-fold or 1000-fold, and rainbow trout in the mesocosms were sampled after 8 weeks. Relative to controls, there was very little difference in MFO activity, with about a 50% induction observable only at the high concentration of the effluent from the mill with traditional bleaching. Lehtinen (1996), in a review of biochemical responses of fish in mesocosms, found that many effluents actually inhibited MFO activity, as well as activity of conjugating enzymes, at concentrations as low 0.25%, likely due to the presence of fatty and resin acids and chlorophenols. The inhibition of enzyme responses raises the possibility that comparisons of responses to technology may be confounded by “false negatives” due to toxicity of effluent components. Despite a large number of studies involving effluents from mills using many different bleaching sequences, there was no strong evidence that bleaching was the source of toxic components in final effluent (Lehtinen, 1996). The MFO responses of rainbow trout (measured as EROD activity) exposed to different whole mill effluents tested in mesocosms are compiled in **Figure 16**. Despite the fact that effluents from modern ECF and TCF processes generally show low or no acute toxicity, the EROD response tends to be lower than that of unexposed fish implying possible enzyme inhibition and sublethal toxicity. It is also noteworthy that peat bog water and urban sewage water show the same tendency.

4.2.3 Laboratory studies - effluents from mills

Kovacs and McGraw (1996) provided a detailed review of laboratory studies of effluent toxicity for studies published between 1991 and 1994. For both acute and chronic toxicity, there was evidence of an improvement in effluent quality, as shown by reduced effects on algae, invertebrates and fish, as mills upgraded pulping, delignification, bleaching, and waste treatment processes. However, the results did not allow clear conclusions about the benefits of specific technologies due to the numbers of changes made simultaneously, and the use of different test species and protocols by different authors. While laboratory tests can contribute to understanding the impacts by providing data from controlled conditions, data can often not be extrapolated to real situations because the tests are over-simplified.

4.2.3.1 MFO Induction

Detailed mill sampling and laboratory bioassays demonstrated that the most potent source of MFO-inducing compounds within a bleached kraft mill, and a large source by volume, was the spent cooking liquor from kraft pulping (Schnell *et al*., 1993). Hence, bleaching is not essential for MFO induction, and inducers appear to be natural products derived from wood (see section 5.1.2). Nevertheless, the bleach plant (50% substitution) and first extraction stage effluents were much less potent MFO inducers than black liquor, but larger sources of inducers by volume. Possible explanations are carry-over of spent pulping liquor to the bleaching stage; a continued release of wood extractives or breakdown products of lignin by bleaching; or enhanced hydrophobicity and increased accumulation of extractives due to chlorination.

Schnell *et al*. (1993) also showed that the potency for MFO induction in fish of a variety of waste streams could be reduced significantly through enhanced biological treatment in lab-scale and pilot-scale treatment systems, specifically by increased solids and hydraulic retention times. However, treatment may simply transfer inducers to sludge. Sludges from the treatment system and downstream sediments of a bleached sulfite mill cause MFO induction in trout in sediment bioassays (Parrott and Hodson, unpublished data), and the highest concentrations of inducers in bleached kraft mill effluents were associated with particulates and colloidal material (Burnison *et al*., 1996). Lehtinen *et al*. (1991) found that sludges incorporated into fish food caused MFO induction in exposed fish, but induction was less when the sludge was pre-extracted with hexane.

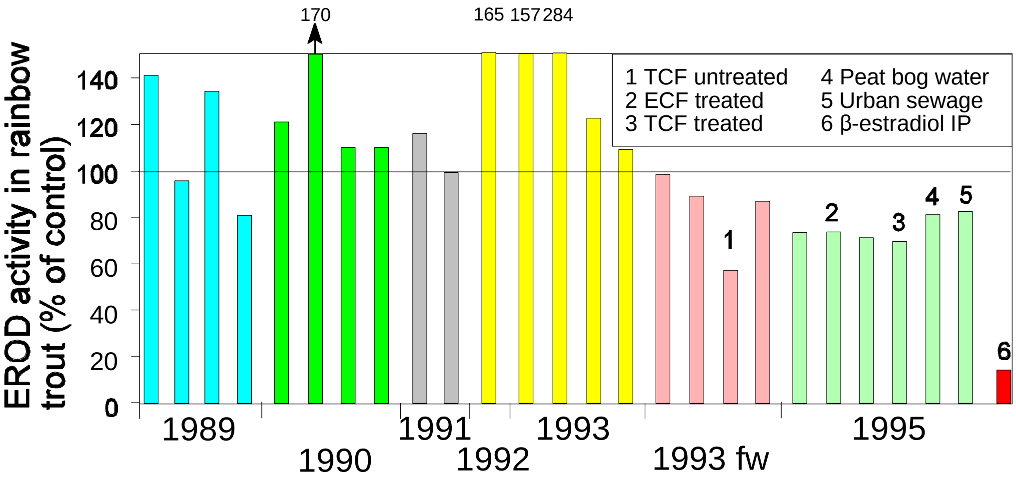


Figure 12 Changes in liver tissue EROD activity in rainbow trout exposed to 1/400 dilution of effluents from pulp mills and other sources.

Effluent from a variety of kraft mills, including those with chlorine bleaching, those with 50-100% substitution, and those with no bleaching at all, caused MFO induction in trout (Williams *et al.*, 1996). Variation in induction among mills was least when differences in water use (effluent dilution) were removed from the comparison by normalizing to DOC. Williams found low coefficients of variation for comparisons of MFO induction potency and concentrations of AOX, chlorophenols, and resin and fatty acids. Coefficients of variation were best for DOC.

Effluent from five pulp mills on the Athabasca River system were surveyed for release of MFO inducing compounds using semi-permeable membrane devices (SPMDs) to collect samples (Parrott *et al*., 1996). SPMDs are 1 m x 2 cm flat tubes of polyethylene that contain 1.0 g of triolein, a fish lipid into which hydrophobic compounds can partition. In laboratory and field studies of two mills in Ontario, SPMDs accumulated significant amounts of MFO-inducing compounds from secondary-treated effluent from BKMs with 50% substitution, as shown by bioassays with mammalian cells in culture (Parrott *et al*., 1994). Induction by extracts of SPMDs paralleled induction in native fish from receiving waters, lab fish caged in receiving waters, and trout exposed to the same effluent in the laboratory. However, in the Athabasca River, none of the SPMDs installed in effluent discharge ponds or pipes, or in the river downstream of each mill, accumulated inducing compounds. In contrast, very high concentrations were accumulated from a "positive control", the effluent of a tar sands extraction facility.

The Athabasca mills included kraft, TMP and CTMP mills, with and without bleaching. Three mills were ECF and two mills were TCF, using alkaline peroxide and hydrogen peroxide as bleaching agents; all had secondary treatment. The absence of inducing compounds shows that pulp and paper mills employing either ECF or TCF bleaching sequences and secondary waste treatment are capable of producing final effluent without MFO inducing compounds. Because only final mill effluent was sampled, the technology responsible for the absence of inducing compounds could not be discerned.

Martel and Kovacs (1996b) tested the effects of different pulp mill technologies on the induction of MFO activity in trout fish by bioassays of effluents from a variety of pulp mills: kraft (N=7), CTMP (N=3), and TMP (N=3). For bleaching, four kraft mills used oxygen delignification and 100% ClO_2 substitution, two had similar systems but did not use delignification, and the last had two pulping lines using partial substitution (OD60C40EoHD and C50D50EHD). The CTMP mills brightened the pulp with hydrogen peroxide, while the TMP mills used hydrosulphite. Over a concentration range of 1-10%, primary-treated effluent caused MFO induction, but the extent of induction was not correlated to the type of pulping, wood furnish, pulp bleaching or brightening; there were no systematic differences in response to effluents between mills that did not use ClO_2 and those that did. Similarly, secondary treatment by aerated lagoons or activated sludge reduced induction relative to untreated effluent, but the extent of reduction was unrelated to the technology of waste treatment. While it is clear that effluents from mills using ECF bleaching caused no greater induction than effluents from mills using no bleaching at all, the relationship of induction to technology remains enigmatic. One reason is that comparisons were based only on the extent of MFO induction in fish exposed to a limited range of test concentrations, some of which approached lethal levels.

4.2.3.2 Chronic toxicity

A series of fathead minnow life-cycle bioassays and 7-d *Ceriodaphnia* tests conducted by PAPRICAN and NCASI (summarized in Table 4-2) demonstrated the benefits of process improvements on the chronic toxicity of BKME and the relative toxicity of TMP effluent. Kovacs *et al*. (1995b) measured the chronic toxicity to fathead minnows of a secondary-treated effluent from a bleached kraft mill pulping mixed softwoods (predominantly lodgepole pine) with modified continuous cooking and bleaching with D/C Eo [DED] after oxygen delignification; ClO_2 substitution was 45%. Dioxins in the effluent were below detection (3 pg/L for 2,3,7,8-TCDD) but tetrachloro, hexachloro, heptachloro and octachloro furans were measurable, with mean concentrations of 2,3,7,8-TCDF of 19 pg/L (ppq). There were no effects on survival and growth of minnows, but reproduction was impaired: there was a delay in sexual maturity and a reduction in the numbers of eggs produced per female. The authors estimated that effects occurred at concentrations of 2.5% (v/v) or greater. *Ceriodaphnia* reproduction was also affected, but at much higher concentrations. The reproductive effects were very similar to those observed in wild fish downstream of kraft mills, with and without secondary treatment, and with and without ClO_2 substitution (Gagnon *et al*., 1995; Munkittrick *et al*., 1994; Adams *et al*., 1992).

Reproduction of fish returned to normal when effluent from the same plant was tested after a variety of mill improvements (Kovacs *et al*., 1996); effluent concentrations impairing *Ceriodaphnia* reproduction increased from 36% to 68% (v/v). Changes included a change to 100% ClO_2 substitution (now D100Eop[DED]), improved brown stock washing, addition of anthroquinone, improved recovery and treatment of condensates, improved steam stripping of volatiles, reduced black liquor losses, a 35% reduction in defoamer use, and a change in type of pitch dispersants. The effluent was treated with primary clarification of all streams except the bleach plant effluents. Where they had previously been treated in a clarifier, they now were combined with primary-treated effluent for secondary treatment in a 5-d retention aerated lagoon. Secondary treatment included the domestic sewage from a town of 9,000, so that ammonia was no longer added to promote treatment. The lagoons were dredged and aeration reduced to increase the quiescent zone and reduce total suspended solids. The final effluent (70,000 m^3/d) was mixed with 35,000 m^3/d of cooling water before discharge. Previously, about 15,000 m^3/d of cooling waters were passed through treatment, while 20,000 m^3/d was by-passed. Now, all cooling waters by-pass treatment and are mixed with final effluent, which now contains a greater proportion of clean cooling water. The concentrations of color, COD, BOD, TSS, and AOX, declined by 30-80%, with the largest change in AOX; no measurements of dioxins and furans were reported. While effluent quality certainly improved, the role of changes in bleaching technology in toxicity reduction could not be assessed because there were too many simultaneous changes in mill operations and effluent treatment. As well, the contribution of urban sewage to toxicity could not be discriminated with these data.

Table 4-2. Results of chronic toxicity tests using effluent from mills with ECF bleaching (adapted from NCASI 1997)

Mill Type	Bleaching sequence[a]	Effluent treatment[b]	Output (ADMT)[c]	Furnish (%)	Water Use m^3/ADMT	Response[d]	IC25[e] (% v/v)	Reference
Kraft - W. Canada - *before* ClO$_2$ substitution	O-D$_{45}$C$_{55}$ E$_o$DED	Clarifier 4-5 d ASB	1100	Pines 60 Spruce 32 Fir 8	98	*Ceriodaphnia*		Kovacs et al 1995b
						Survival	>100	
						Reproduction	36	
						Fathead minnow		
						Percent hatch	>10	
						Growth - life cycle	>20	
						Survival - life cycle	>20	
						Time to maturity	10	
						Sex ratio	5.0	
						Egg production	1.7	
Kraft - W. Canada - *after* ClO$_2$ substitution and numerous other process changes	O-D$_{100}$E$_{op}$ DED	Clarifier 5 d ASB	1100	Pines 55 Spruce 40 Fir 5	95	*Ceriodaphnia*		Kovacs et al 1996
						Survival	>100	
						Reproduction	68	
						Fathead minnow		
						Percent hatch	>20	
						Growth - life cycle	>20	
						Survival - Life cycle	>20	
						Time to maturity	>20	
						Sex ratio	>20	
						Egg production	>20	
						GSI, egg diameter	>20	
						Histopathology	>20	
Kraft - SE USA - *before* ClO$_2$ substitution	CEHDED	Clarifier 14 d ASB	700	hardwood 25 pine 75	150	*Ceriodaphnia*		NCASI 1985
						Survival	>100	
						Reproduction	42	
						Fathead minnow		
						Percent hatch	98 - >100	
						Larval growth - 56 d	10- 24	
						Larval Survival - 56 d	10 - 91	
						Egg production	8 - 14	

Mill Type	**Bleaching sequence**[a]	**Effluent treatment**[b]	**Output (ADMT)**[c]	**Furnish (%)**	**Water Use m^3/ADMT**	**Response**[d]	**IC25**[e] **(% v/v)**	**Reference**
Kraft - SE USA - *after* ClO$_2$ substitution	O-W-DE$_{op}$-W-DE$_o$D	Clarifier 14 d ASB	700	hardwood 25 pine 75	150	*Ceriodaphnia*		NCASI 1996
						Survival	>100	
						Reproduction	50	
						Fathead minnow		
						Percent hatch	>100	
						Larval growth - 56 d	>100	
						Larval Survival - 56 d	92 - >100	
						Egg production	52 - 61	
						GSI, egg diameter	>100	
						Steroid synthesis *in vitro*	>100	
Kraft	D-E$_{op}$-D-E$_p$-D	Clarifier 5 d ASB	2200	hardwood 10 pine 90	60	*Ceriodaphnia*		NCASI 1997
						Survival	93	
						Reproduction	50	
						Fathead minnow		
						Percent hatch	26 - >100	
						Larval growth - 56 d	81 - >100	
						Larval Survival - 56 d	54 - >100	
						Egg production	18	
						GSI, egg size	89 - >100	
						Steroid synthesis *in vitro*	35	
						LSI, CF, MFO induction	100 - >100	
Kraft - E. Canada	O-D$_{55}$C$_{45}$E$_o$HD O-E$_{80}$D$_{20}$E$_o$HD O-C$_{80}$D$_{20}$EHD	Clarifier 7 d ASB	943	Hardwood 45 Softwood 55	105	Fathead minnow		Robinson 1994; Kovacs and McGraw 1996
						Percent hatch	>50	
						Larval growth - 56 d	>50	
						Larval Survival - 56 d	>50	
						Time to maturity	50	
						Egg production	12	
						GSI	>50	
						Steroid synthesis *in vitro*	25	
						LSI, CF	>50	

Mill Type	**Bleaching sequence**[a]	**Effluent treatment**[b]	**Output (ADMT)**[c]	**Furnish (%)**	**Water Use m^3/ADMT**	**Response**[d]	**IC25**[e] **(% v/v)**	**Reference**
TMP - W. Canada	hydrosulfite bleaching	Clarifier 2-2.5 AS	600	Spruce 56 Pines 36 Balsam fir 8 Recycled fibre <8	25	*Ceriodaphnia*		Kovacs *et al.*, 1995a
						Survival	>100	
						Reproduction	65 - >100	
						Fathead minnow		
						Percent hatch	>20	
						Growth - life cycle	>20	
						Survival - Life Cycle	>20	
						Time to maturity	>20	
						Egg production	>20	
						Histopathology	>20	

[a] Bleaching sequence codes: D-chlorine dioxide; O-oxygen delignification; H-hypochlorite; E-extraction; E_{op}-extraction with oxygen and peroxide; C-chlorine; W-washing;

[b] ASB - Aerated Stabilization Basin; AS - Activated Sludge

[c] ADMT - Air Dried Metric Tonne

[d] Response -Where 7 d, 28 d, 56 d and complete life cycle tests were run, the range of concentrations causing changes in mortality and growth are given, not the values for each bioassay.

[e] IC25 - Effluent concentration causing a 25 % change in response; where the IC25 was not available, the lowest concentration causing effects was listed.

A similar comparison of effluent effects on *Ceriodaphnia* and fathead minnows was made by NCASI (1985; 1996; Borton and Bousquet 1996). A mill in the S.E. United States converted from chlorine bleaching to 100% ClO_2 substitution and oxygen delignification in concert with a variety of other process changes. The result was a reduced discharge of chlorinated compounds, BOD, TSS, color and conductivity but no change in 7-d toxicity to *Ceriodaphnia* reproduction or survival and growth of larval fathead minnows. However, chronic toxicity of effluent to fathead minnows was reduced considerably. Over 28-56 days, growth and survival of juveniles were unaffected by 100% post-conversion effluent, in contrast to pre-conversion effluent that caused significant impairment at concentrations of 10% (v/v). In life cycle tests, egg production of fathead minnows was actually enhanced by post-conversion effluent at concentrations above 10% (v/v), and there were no effects of 100% effluent on other reproductive responses such as gonad size, steroid synthesis, and egg diameter. MFO induction was apparent, as indicated by CYP1A protein concentrations, but enzyme activity showed little change. However, fathead minnows have high background MFO activity, relative to trout, and they do not show marked induction, even when exposed to known inducing compounds (Lewis 1997). Overall, while these results correspond to those of Kovacs *et al.* (1995b; 1996), the study does not provide a good basis for judging the benefits of any one technology. Too many changes were made between the “before” and “after” studies to understand which made the greatest contribution to improved effluent quality.

The toxicity of secondary-treated effluents from two bleached kraft mills was tested after introduction of oxygen delignification before bleaching by 60-70% ClO_2 (Hall *et al.*, 1996). Toxicity to various marine and freshwater invertebrates and fish was low, both before and after the process changes, so there appeared to be little change in toxicity, despite large changes in the discharge of chlorophenols and AOX.

The toxic effects found in the “before” and “after” studies by PAPRICAN and NCASI are similar to those of two other studies, one a mill before conversion to ECF, and one after conversion. Delayed sexual maturity and reduced egg production were found in fathead minnows exposed to secondary-treated effluent from a kraft mill using 50% substitution (Robinson, 1994; Table 4-2), with effluent toxicity similar to that of the pre-conversion study by NCASI (1985) and intermediate between the pre- and post-conversion studies by Kovacs *et al.* (1995a; 1996). While there seems to be general agreement among these studies of effects, toxicity, and the benefits of mill improvements, there is still variability among studies with respect to the concentrations of effluents causing specific effects. In fact, the concentrations causing reproductive impacts on fathead minnows in life-cycle tests at a mill before chlorine dioxide substitution (NCASI 1985; Table 4-2) appear little different than concentrations of effluent from another mill after chlorine dioxide substitution (NCASI 1997, Table 4-2). However, as explained in NCASI (1997), much of this difference can be accounted for by water use. As indicated in Table 4-2, water use per ADMT of pulp varied by 2.5 fold among mills studied, and when taken into account, the concentrations of effluent after conversion appear less toxic than before conversion, even when comparing among mills. The improvements in comparability among studies after applying a simple normalizing parameter such as water use indicates that other normalizing techniques should be attempted. After normalizing to water use, there is also quite good agreement for results of studies of three ECF mills (Kovacs et al 1996; NCASI 1996; NCASI 1997; Table 4-2).

Compared to ECF effluents, less toxicity was observed with a secondary-treated TMP effluent (Kovacs *et al*., 1995a; Table 2). The mill produced about 600 T/d of hydrosulfite-bleached pulp from mixed western softwoods (predominantly white and black spruce) and about 8% recycled fibre. Effluent was clarified, passed through an equalization basin (9-12 h retention) and a secondary activated-sludge plant with 2-2.5 d retention. The effluent was not acutely toxic; i.e., LC50s for fathead minnow and *Ceriodaphnia* exceeded 100% effluent. Similarly there were no effects on 7-d growth and survival of fathead minnow larvae and only slight effects on 7 d *Ceriodaphnia* reproduction (1 sample in 4 caused effects at 65% (v/v)). During 200 d fathead minnow life cycle tests, there were no effects on mortality and growth (eggs through adults) at the maximum concentration tested (20% v/v), nor on sex ratios, time to maturity, reproductive activity or production of eggs. Samples taken for histopathology showed no unusual lesions. It was obvious from these tests that the "quality" of TMP effluent was greater than that of the kraft effluents. However, the reasons for higher quality are difficult to discern, because there were so many differences among the mills in technology and operation.

A battery of toxicity tests were applied by Ahtiainen *et al*. (1996) to whole effluents from mills using TCF, ECF and conventional chlorine bleaching; effluent was tested before and after secondary treatment. The effluents were derived from two BKMs bleaching hardwood and softwood pulps. Secondary treated effluents were non-toxic to bacteria (2 species), algae, *Daphnia magna*, and zebra danio embryos and larvae. Untreated effluents were sometimes toxic, with no differences in toxicity to *Daphnia magna* among the effluent types. Similar results were observed with the embryo-larval assay, although TCF and conventional untreated effluents from pine pulping were most toxic. Generally speaking, variability was so great that no clear conclusions emerged about the relative toxicity of the three effluents. This was true whether toxicity was expressed as percent effluent, toxic units or toxicity emission factors, although one untreated ECF effluent from hardwood pulping was exceptionally toxic. BOD, COD and TOC were all strongly correlated to toxicity (Verta *et al*., 1996), and correlations of toxicity to concentrations of total phenols, fatty acids and resin acids could explain 79% of the variation in toxicity; AOX was less well correlated to toxicity. The conclusion of this study was that natural wood constituents accounted for most of the toxicity and that their concentrations were unrelated to ECF or TCF bleaching.

Ten of 15 bleached kraft mills studied by Priha (1996) used ECF bleaching and all employed some type of treatment. The exceptions to the ECF bleaching were TMP (N=1), conventional bleaching (N=1), combined ECF and TCF (N=2), and combined ECF and conventional bleaching (N=1). None of the mill effluents were toxic to *Daphnia*, and all secondary-treated effluents were non-lethal to zebrafish embryos, in contrast to effluents receiving only primary treatment. Only two of the effluents caused EROD induction in isolated rainbow trout cells, but seven were inhibitory. Correlations of toxicity to bleaching technology were not possible because none of the effluents was particularly toxic.

Recent data by Lovblad *et al.* (1994) on the toxicity of ECF and TCF effluents to Microtox^{TM}, *Selenastrum* and Zebrafish (*Danio rerio*) suggested that toxicity emission factors were significantly reduced by TCF relative to ECF bleaching. However these conclusions were unjustified because all effluents had very low toxicity. Because toxicity was close to or greater than 100% effluent, relative toxicity, based on toxicity emission factors, was a measure of effluent flow rather than toxicity.

In summary, the primary ways to reduce effluent effects appear to be the prevention of black liquor losses and the provision of adequate secondary treatment. Despite the numerous studies cited above, there is little evidence that ECF bleaching is responsible for effects on fish. Some of the variation in toxicity among mills, or after mill improvements, might be more understandable if water use (i.e., dilution of chemical constituents) was normalized by reference to factors such as COD, BOD, TSS and TOC (e.g., Williams *et al.*, 1996). Expressing induction potency or toxicity in mass loading terms (e.g., Schnell *et al.*, 1993; Lovblad *et al.*, 1994) might also be a better indicator of which processes release inducers or toxicants, how much is released, and whether they are recovered during waste recycling and effluent treatment (Hodson *et al.*, 1996b). The likely transfer of inducers to particulates and sludges during treatment means that a mass-balance based on both liquids and solids must be calculated for mill-wide or technology-specific comparisons, and this principle would also apply to substances causing reproductive effects.

This review has focused on the relationship between pulp mill technologies and effects of pulp mill effluents, with little regard for the chemical composition of those effluents. Previous reviews (Solomon *et al.*, 1993; Hodson *et al.*, 1996a) have demonstrated that effects cannot be related to AOX, and Williams *et al.* (1996) have shown the benefit of normalizing effects to DOC concentrations to account for dilution. Beyond these relationships, there are no measurements of effluent chemistry reported in studies of effects that provide a perspective on the role of technology. Concentrations of BOD, COD, TSS, phenols and resin acids are unrelated to the observed effects. With the developing understanding of the mechanisms of effects, and of the compounds associated with effects (e.g., retene, sitosterol, juvabione), future studies can employ more meaningful measurements of effluent chemistry. In fact, the study by Martel *et al.* (1996b) demonstrated clearly the close association between MFO induction and juvabione concentrations in different effluents of one pulp mill.

Studies of effluent toxicity to fish and invertebrates, and of MFO induction in fish, suggest that pulping is the most important source of substances causing toxicity. These would likely be wood extractives or breakdown products of lignin. While there are clear benefits of black liquor spill control and of secondary treatment, no studies demonstrated environmental advantages of TCF bleaching compared to ECF bleaching. There were also no studies that established a clear link between toxicity reduction and the installation of any other specific technologies. Improvements in mill effluents were associated with multiple changes to many aspects of mill operations, and could not be ascribed to any one technology. Future advances in *this area rely on a mass balance approach that accounts for both liquid and solid wastes, and on the measurement of the compounds associated with effects.*

4.2.4 Laboratory studies - experimental effluents

Cates *et al*. (1995) used lab-scale bleaching of hardwood and softwood pulp (TCF sequence, pine: xylanase (X), DTPA (Q), Ep, Ozone (Z) and peroxide (P); eucalyptus: XZQP) (ECF, hardwood and softwood: DEDED). Bioassays of 15-minute Microtox at pH 6.3 gave EC50s ranging from 0.24% effluent (ECF) to 0.56% (TCF). Treatment of effluent with white rot fungus reduced toxicity to >1.0% effluent, the highest concentration tested. Consequently, the effect of bleaching on the toxicity of final treated effluent was unknown.

O'Connor and Nelson (1996) tested the toxicity of effluents from laboratory bleaching of pulps using ECF (OD100Eop) and TCF (OQP) sequences. Bioassays included acute lethality to Microtox, *Ceriodaphnia*, and *Daphnia* and reproductive effects on *Ceriodaphnia*. The acute lethality of the ECF lab bleach effluent was similar to that of a real ECF mill effluent (100 and 87% v/v respectively), as were reproductive toxicity thresholds (53 and 56% v/v respectively). The acute lethality of TCF bleaching effluents from four wood species varied considerably. Although non-toxic to microtox bacteria, the LC50s for *Daphnia* and *Ceriodaphnia* varied from about 50% to >100% (v/v), depending on whether cedar (most toxic), hemlock, douglas fir, or spruce/pine/fir (least toxic) were being tested. In all cases, effluents were quenched with metabisulfite to avoid bias due to residual peroxide. These effluents were also toxic to *Ceriodaphnia* reproduction, with threshold concentrations ranging from 4.7 to 13% v/v; the differences among wood species were less pronounced. By comparison, the spruce/pine/fir effluent from ECF bleaching was about 10-fold less toxic (53% v/v) than effluent from TCF bleaching (5.1% v/v). These tests were of bleaching effluent only and did not determine whether treatability to remove toxicity in final effluent would vary with the type of bleaching used.

Effluents generated in the laboratory from ECF bleaching sequences were either equally toxic or less toxic than effluents from TCF bleaching sequences. However, because experimental designs were incomplete, the effect of bleaching on treatability, and hence on the toxicity of final effluents, was not evaluated. Studies since 1993 have identified natural compounds causing MFO induction and impaired regulation of steroid hormones in fish. However, few studies directly addressed the question of the role of bleaching technologies. There was no compelling evidence that effluents from pulp mills using ECF bleaching are any more or less toxic, or cause any more or less MFO induction than those from mills using TCF bleaching. Progress in this area requires a mass balance approach and chemical analysis of effluents to understand relative toxicities of effluents from different mills and technological processes. The importance of sludges, particulates, and dissolved organic carbon as a source of compounds affecting fish has not yet been properly evaluated.

5 CHARACTERIZATION OF RISK

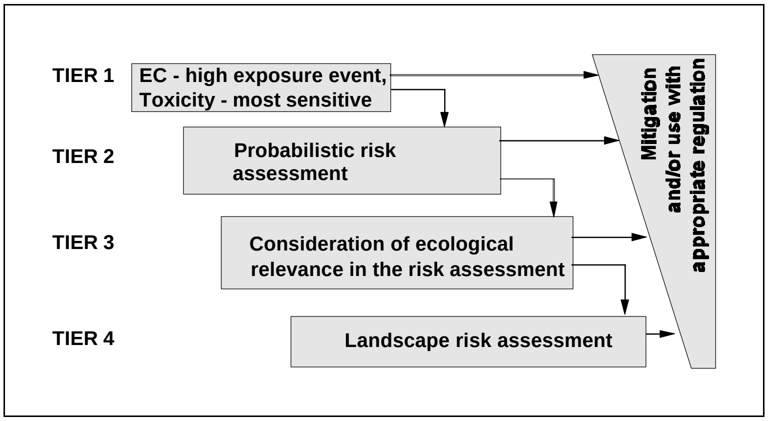


Figure 13 Illustration of the tiers of risk assessment (adapted from SETAC 1994).

Characterization of possible responses to exposures at the level of the organism has traditionally been conducted by comparison of the concentration of the stressor/s found in the environment to the responses reported for that stressor/s in the laboratory or the literature. This process is normally conducted in a series of tiers, starting with conservative assumptions and progressing to more and more realistic assessments (**Figure 13**). The initial use of conservative criteria allows substances that truly do not present a risk to be eliminated from the risk assessment process, thus allowing the focus of expertise to be shifted to more problematic substances. In the Report of the Aquatic Risk Assessment Dialogue Group (SETAC, 1994), it was suggested that four tiers be used in the risk assessment process for pesticides in aquatic ecosystems. These tiers began with a simple “worst-case” estimation of environmental concentration which was compared with the effect level for the most sensitive organism (the quotient approach). If the result of this comparison suggested no hazard, no regulatory action would be needed. If the result suggested a potential risk, further tiers of risk assessment with more realistic and more complete exposure and effects data could be applied to the problem.

The first tier in risk characterization process is the use of hazard quotients. These are simple ratios of exposure and effects and may be used to express hazard or relative safety. For example:

$$Hazard \approx \frac{Exposure\ \ concentration}{Effect\ \ concentration} \qquad or\ \ Margin\ \ of\ \ Safety \approx \frac{Effect\ \ concentration}{Exposure\ \ concentration}$$

The calculation of quotients traditionally has been conducted by utilizing the susceptibility of the most sensitive organism or group of organisms and comparing this to the greatest exposure concentration. This may be made more conservative by the use of a safety factor to allow for unquantified uncertainty in the effect and exposure estimations or measurements.

The quotient model for ecological risk assessment fails to explicitly consider ranges of sensitivity in different species in the ecosystem, or ranges of concentration. Uncertainty factors are an attempt to account for these ranges but they may be excessively conservative or not conservative enough. Expressing the results of an exposure or effects characterization as a distribution of values in a probabilistic approach rather than a single point estimate has the advantage of using all relevant single species toxicity data and, when combined with exposure distributions, allows quantitative expression of risks to communities of organisms (Health Council of the Netherlands, 1992; SETAC, 1994).

Probabilistic approaches to assessing and managing risks are not new. The probability of occurrence of a particular event is widely used in the characterization of risks from many physical and medical events in humans and for reduction in failure rate in mechanical and civil engineering projects (time between failures, one-in-one- hundred-year floods, etc.). Many of the issues in probabilistic risk assessment have been the subject of a recent journal debate (Power and McCarty, 1996; Anderson and Yuhas, 1996; Burmaster, 1996; Richardson, 1996). The extension of this concept is now being applied in ecological risk assessment for the characterization of both exposures and effects (Solomon *et al*., 1996; Klaine *et al*., 1996; Solomon, 1996).

5.1 ASSESSMENT OF CHLORINATED PHENOLICS SUBSTANCES IN PULP MILL EFFLUENT.

Mills routinely analyze effluents for the presence of certain chlorinated compounds including the phenols and PCDD/PCDFs (see section 2). These data were used for a risk characterization conducted using a tiered approach as described above. A tier-one hazard characterization was conducted by comparison of the highest concentration of the substance reported from analytes of effluents from a number of mills using 100% chlorine dioxide (data collected from 1990 to the present) to the responses reported for that substance in the literature (NCASI, 1992). As several criteria are used to assess toxicity, these comparisons were treated differently. Where chronic toxicity was measured, a margin of safety of 10 was used, while, for acute toxicity a margin of safety of 100 was used. In the absence of chronic toxicity data, a MOS of 100 or more for an acute test may be considered protective of chronic responses (Sloof *et al.,* 1986).

Only data for mills using 100% chlorine dioxide substitution were used in this hazard assessment. For some chlorinated phenols, concentrations above the limit of detection were not reported. These substances were: 2,3,4,5-Tetrachlorophenol (N = 26), 2,3,4,6-Tetrachlorophenol (N = 60), 2,3,4-Trichlorophenol (N = 17), 2,3,5,6-Tetrachlorophenol (N = 16), 2,3,5-Trichlorophenol (N = 16), 2,4,5-Trichlorophenol (N = 43), 2-Chlorophenol (N = 33), 3,4,5 Trichlorosyringol (N = 13), 3,4-Dichloroguaiacol (N = 5), 4,5-Dichloroveratrole (N = 39), 5,6-Dichlorovanillin (N = 24), Pentachlorophenol (N = 79), and Tetrachloroveratrole (N = 37). For those where concentrations above the limit of detection were reported, the highest concentration was compared to several toxicity endpoints (NCASI, 1992) as shown in Table 5.1. Only two of these substances (tetrachloroguaiacol and tetrachlorocatechol) produced margin of safety quotients less than the chosen criteria (100). Risks from these substances were assessed using a probabilistic approach.

Table 5.1 Toxicity criteria and hazard quotients for chlorinated compounds in mill effluents produced from bleaching processes with 100% chlorine dioxide substitution

Substance	Species	Response criterion	Response concentration (RC) µg/L	Highest environmental concentration reported (EC) µg/L	RC/EC	Hazard	Hazard Criterion	N
2,4-Dichlorophenol	Rainbow trout fry	96 h LC50	2600	0.4	6500	low hazard	100	34
	FW Biota	EPA acute criterion	2020	0.4	5050	low hazard	10	
	FW Biota	EPA chronic criterion	365	0.4	913	low hazard	10	
2,4,6-Trichlorophenol	Rainbow trout	96 h LC50	730	1.5	487	low hazard	100	79
4-Chloroguaiacol	Zebrafish	Egg/larvae NOEC	700	1.2	583	low hazard	10	38
4,5-Dichloroguaiacol	Rainbow trout	96 h LC50	2200	0.3	7333	low hazard	100	39
	Zebrafish	Egg/larvae NOEC	300	0.3	1000	low hazard	10	
3,4,5-Trichloroguaiacol	Rainbow trout	96 h LC50	750	4	188	low hazard	100	70
	Zebrafish	Egg/larvae NOEC	200	4	50	low hazard	10	
4,5,6-Trichloroguaiacol	Rainbow trout	96 h LC50	800	1.6	500	low hazard	100	65
	Zebrafish	Egg/larva NOEC	300	1.6	188	low hazard	10	
Tetrachloroguaiacol	Rainbow trout	96 h LC50	300	3.7	81	some hazard	100	79
	Zebrafish	Egg/larva NOEC	200	3.7	54	low hazard	10	
4-Chlorocatechol	Zebrafish	Egg/larva NOEC	700	0.3	2333	low hazard	10	33
3,4-Dichlorocatechol	Salmon (*O. nerca*)	96 h LC50	2700	0.1	27000	low hazard	100	38
	Algae (*S. capricornutum*)	EC50	1800	0.1	18000	low hazard	100	
3,5-Dichlorocatechol	Zebrafish	Egg/larva NOEC	500	0.1	5000	low hazard	10	38
4,5-Dichlorocatechol	Rainbow trout	96 h LC50	100	1	100	low hazard	100	39
	Zebrafish	Egg/larva NOEC	500	1	500	low hazard	10	
3,4,5-Trichlorocatechol	Rainbow trout	96 h LC50	1000	2.5	400	low hazard	100	75

Table 5.1	**Toxicity criteria and hazard quotients for chlorinated compounds in mill effluents produced from bleaching processes with 100% chlorine dioxide substitution**							
Substance	**Species**	**Response criterion**	**Response concentration (RC) µg/L**	**Highest environmental concentration reported (EC) µg/L**	**RC/EC**	**Hazard**	**Hazard Criterion**	**N**
	Zebrafish	Egg/larva NOEC	200	2.5	80	low hazard	10	
3,4,6-Trichlorocatechol	Brown trout	96 h LC50	900	1	900	low hazard	100	65
	Zebrafish	Egg/larva NOEC	350	1	350	low hazard	10	
Tetrachlorocatechol	Rainbow trout	96 h LC50	400	5.8	69	some hazard	100	77
	Zebrafish	Egg/larva NOEC	150	5.8	26	low hazard	10	
Trichlorosyringol	Zebrafish	LOEC	800	0.36	2222	low hazard	10	23
4,6-Dichloroguaiacol			No data	0.2				33
3,4,6-Trichloroguaiacol			No data	0.3				53
6-Chlorovanillin			No data	2				6
3,4,5-Trichloroveratrole			No data	5.8				36

Measured exposure concentrations for tetrachloroguaiacol and tetrachlorocatechol were ranked and percent plotting position calculated as reported in Solomon *et al.* (1996). These ranks were plotted against log of concentration (**Figure 14**). The most sensitive toxicity data (Table 5.1) were plotted on the graph. Given similar circumstances (100% chlorine dioxide substitution and the same processes in the mill) and from the graph, it is clear that the likelihood of a concentration greater than the toxicity criteria for tetrachloroguaiacol and tetrachlorocatechol occurring in the effluent is very low (less than 1%). Accordingly, the risk is judged to be so low as to be negligible.

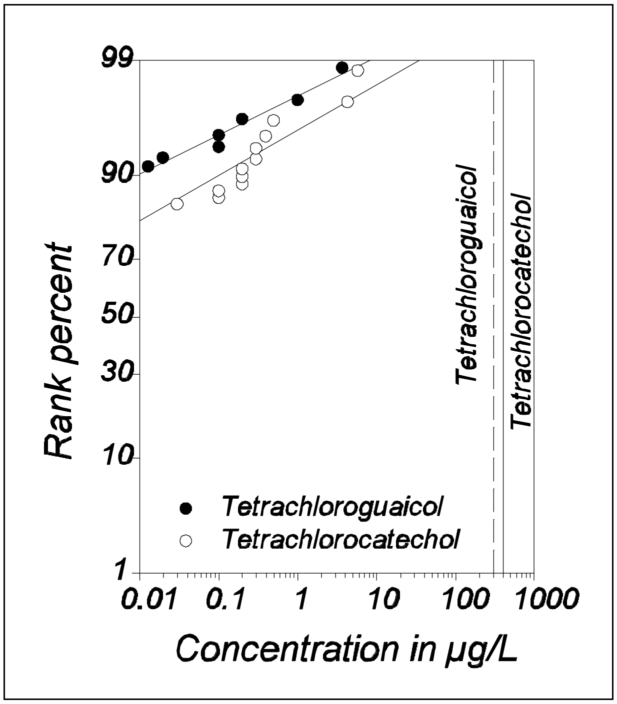


Figure 14 Distributions of concentrations of tetrachloroguaiacol and tetrachlorocatechol from mills using 100% chlorine dioxide substitution compared to the most sensitive toxicity endpoint (vertical lines).

Chlorophenols are generally believed to act through a common mechanism of action — uncoupling of oxidative phosphorylation. As a result, it has been assumed that the toxicity of mixtures of two or more chlorophenols will interact additively. Kovacs *et al.* (1993) used toxicity data on freshwater and saltwater organisms to develop toxicity equivalency factors (TEFs) for chlorinated phenols in bleached kraft mill effluent. These TEFs can be used to calculate toxicity equivalencies (TEQs) using the toxicity of the most toxic chlorophenol, pentachlorophenol, as a means of converting the potency of components in a mixture into a common unit for assessment purposes. The TEFs that they estimated were:

Pentachlorophenol	1
Tetrachlorophenols	0.5
Trichlorophenols	0.2
Dichlorophenols	0.1
Monochlorophenols	0.04

Many chlorophenols cooccur in the effluents of pulp mills using ECF bleaching. Using these measured concentrations, and the TEFs from Kovacs *et al.*, (1993), it was possible to combine the potency of these substances and express this as a pentachlorophenol TEQ (although pentachlorophenol itself was not detected in any of the effluents). These TEQs were based on measured co-occurrences and thus, when analyzed using the above distributional approach (**Figure 14**), can be used to assess the probability that total potency of the mixture will exceed an assessment criterion in the same way as distributional analysis was used to assess tetrachloroguaiacol and tetrachlorocatechol above. Because the data were collected over space (different mills) and time, the analysis is representative of all 100% ECF mills.

Results of this analysis (**Figure 15** and Table 5.2) show that, even when the combined potencies of the chlorophenols are considered, the likelihood that the EPA FW chronic or acute criteria will be exceeded is low (Table 5.2). This does not suggest an ecologically significant risk. From the data in **Figure 15**, it would appear that the likelihood of exceedence in the post-1993 data set is slightly greater than the pre-1993 data set. This may be an artifact; the method used for calculating the plotting positions is responsive to the number of data points and is more conservative when fewer data are available. The availability of additional data would improve the usefulness of this analysis.

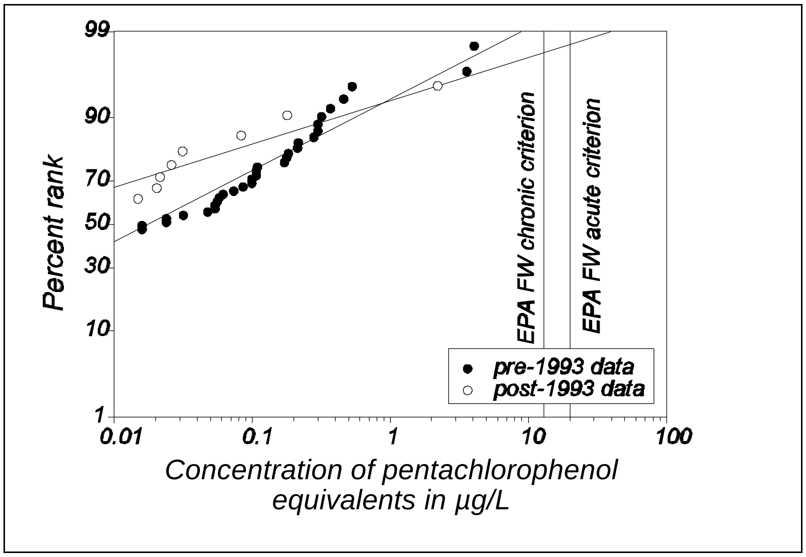


Figure 15 Distributions of concentrations of all chlorinated phenols from mills using 100% chlorine dioxide substitution expressed as pentachlorophenol TEQs and compared to assessment criteria for pentachlorophenol.

Table 5.2 Probabilistic assessment of chlorophenol TEQs exceeding the EPA FW criteria

Data source	y = ax + b			Percent probability of exceeding EPA FW criterion	
	a	b	r^2	Acute	Chronic
Pre-1993	0.86	6.51	0.92	0.5	0.7
Post-1993	0.52	6.49	0.91	1.5	1.9

This approach could be used for other substances found in pulp mill effluent, however, with the exception of the PCDD/PCDFs, these substances are not routinely analyzed for and insufficient data are available for a distributional analysis. Because of the strong historical focus on chlorinated substances, toxicity data for these other, mostly nonchlorinated, compounds are also rare. Distributional analyses of toxicity data for PCDD/PCDFs are complicated by the high bioaccumulation potential of these substances. Thus, acute toxicity tests are less useful as they do not consider the maximal exposure that would result from longer exposures such as may occur in the environment. Distributional analyses could be applied to the concentrations of these substances in effluent or in the receiving waters.

Analysis of additional data on concentrations of chlorinated phenolic substances from mills using 100% chlorine dioxide since 1993 still shows that many of these substances are not detected in the effluents at all. Exposure concentrations of those substances that were detected in mill effluent were generally low and do not suggest that acute and chronic effects from individual substances are likely to result. Assessment of combined toxicity from all chlorinated phenols does not suggest ecologically relevant risks. This observation is a continuation of a trend observed in the previous review.

6 ECOLOGICAL RELEVANCE

6.1 RESPONSES OF POPULATIONS AND COMMUNITIES

As indicated in the previous report (Solomon *et al*., 1993), effluents from some pulp and paper mills have yielded measurable responses by biota in receiving aquatic systems. Considerable information has been published on the effects of effluents from a variety of mills on many species and communities in receiving aquatic systems. However, synthesis of this information is often hampered due to inadequate characterization of the effluent (e.g., pulp source, pulping process, bleaching process, yield, and wastewater treatment are some factors that can greatly influence the presence or magnitude of effects and these factors are often not specified). Further, characteristics of the receiving aquatic system such as flow, volume, extant biota, substrate type and water quality are similarly often not specified. Regardless of these impediments to understanding underlying causes of responses, we are clearly left with some responses deserving consideration and even further study. As for the other sections of this report, this review concentrates on information produced since 1993.

Characterization of known or potential risks currently associated with pulp and paper mill effluents requires careful consideration of responses of receiving aquatic systems. Ecosystem, community, population, organismal and sub-organismal responses may be important endpoints or measurements in this characterization. Also crucial would be changes in these responses spatially and temporally as a function of changes in bleaching processes, wastewater treatment, or other factors that may alter exposures and concomitantly reduce risk in aquatic systems. Measurements of these effects have focused on important components of aquatic systems including fish, invertebrates, plants (algae) and suborganismal responses or biomarkers.

6.1.1 Fish

Since physiological responses are not typically linked with effects on growth, reproduction, or mortality of fish in receiving systems, this section focuses on organismal and population responses to pulp and paper mill effluents. Physiological or sub-organismal responses are considered in a subsequent section of this report. Although it is probably more a phenomenon than an effect, bioconcentration/bioaccumulation is also covered in this section.

Effects of pulp and paper mill effluent exposures on fish have been documented in both wild fish and in life cycle laboratory studies. Decreased reproductive performance in fish has been attributed to changes in maturation and fecundity (Hodson *et al*., 1992a; Gagnon *et al*., 1994a; 1994b; Sandström, 1994). Exposures to effluent from some bleached kraft mills have resulted in an increased age to maturity, smaller gonads and lower fecundity with age in both males and females. Other observations included absence of secondary sex characteristics in males, and females failed to show an increase in egg size with age.

Some field observations of bleached kraft mill effluent effects have been confirmed by laboratory studies. Fathead minnows (*Pimephales promelas*) were exposed throughout their life cycle to secondary-treated bleached kraft mill effluent and responded with delayed sexual maturity, decreased egg production, depressed secondary sexual characteristics, and depressed hormone production. These observations were based on exposures of greater than 20% effluent (McMaster *et al.,* 1996b; Robinson, 1994)

It is important to note that these reproductive responses of fish have not been observed at all pulp mill sites. Minimal changes in reproductive steroids of whitefish and suckers were observed in studies conducted in the effluent of a secondary-treated bleached kraft mill. Only mixed function oxygenase (MFO) induction was observed. In other studies of secondary-treated bleached kraft mill effluent after installation of chlorine dioxide substitution (Swanson *et al*., 1994; Kloepper-Sams, *et al.,* 1994), MFO elevation was the only consistent response observed and numerous other physiological parameters were unresponsive.

Some recent reports have suggested that some previously unrecognized effects were caused by long term exposures to pulp and paper mill effluents. Smits *et al*.(1996a; 1996b) reported evidence of suboptimal immune function in mink (*Mustela vison*) associated with BKME exposure. In this study, mink were exposed via diet (fish from BKME receiving waters) as well as by ingestion of wastewater (25% BKME). This effect has not been field verified, however. Other researchers (e.g., Rao *et al*., 1994; Rao *et al*., 1995) have found some evidence of mutagenic and genotoxic potential of pulp mill effluent in laboratory tests while Metcalfe *et al*. (1995) reported some mutagenic activity of BKME extracts but that BKME is not a potent liver carcinogen in fish. It is important to note that these are laboratory studies with unmeasured exposures and the environmental significance of the results need to be evaluated. In fact, a thorough histopathological study of fish exposed to BKME *in situ* revealed no incidence of cancers in fish (Deardorff *et al*., 1995).

6.1.2 Invertebrates

A thorough study did not find any effects of mill effluents from a variety of sources on reproduction of *Ceriodaphnia*. Receiving waters from a variety of mills using diverse pulping processes and wastewater treatment tactics and discharging >50,000 m^3 day-1 of effluent were studied. The mills included in the study used chlorine/chlorine dioxide bleaching and an unbleached sulfite process which produced unbleached pulp. *Ceriodaphnia* reproduction results (7-d survival and reproduction tests) correspond with the presence of secondary treatment. In all cases of mills with secondary treatment, *Ceriodaphnia* reproduction was enhanced relative to the corresponding reference water. This enhancement is commonly seen when ambient food (e.g., microbes) in the receiving water is increased.

In a laboratory study, Higashi *et al*. (1992) reported a high molecular weight component of bleached kraft mill effluent resembling lignin decomposition products affected the sperm of an echinoderm, the purple sea urchin (*Strongylocentrus purpuratus*). Successful reproduction was apparently prohibited when the effluent material bound to the sperm and prevented recognition of chemical signals from the egg. This effect has not been documented in any field study, however.

6.1.3 Plants (algae)

Previous studies had illustrated that the effects of bleached kraft mill effluent on primary producers in receiving aquatic systems were limited to areas where light penetration sufficient to drive primary production was diminished. This usually constitutes a few meters of a mixing zone where receiving system flows are significant. Some recent reports suggested that chlorine-dioxide based bleaching processes could yield residual chlorate that could be toxic to certain algae in receiving systems. These effects should be most pronounced in systems with low ambient nitrogen concentrations (~ 10 mg NO_3^- N/L) since chlorate toxicity increases with decreased available nitrogen (Van Wijk and Hutchinson, in press). A recent review and research publication by Van Wijk and Hutchinson (in press) concluded that marine macro brown algae (e.g., *Fucus* sp.) are extremely sensitive to chlorate with adverse long-term effects at concentrations of 5-15 mg ClO_3^-/L at low ambient nitrogen concentrations and six month exposures. This is several orders of magnitude more sensitive than other algal species tested. In recent studies of Western Canadian rivers, growth rates and taxonomic composition of algae were not altered by exposures to chlorate up to 500 mg/L at 10 mg/L NO_3^-N (Perrin and Bothwell 1992). In most situations where chlorate would occur, available nitrogen should be protective of algae. To impart some perspective of risk to this situation, it is helpful to recognize that chlorate in effluents from ECF mills are nondetectable at mg/L concentrations. Therefore, a clear margin of safety is apparent, consonant with no quantifiable risk.

An issue that is currently unresolved regarding potential effects of new bleaching processes on primary producers is the toxicity of chelators to algae. Algae are relatively sensitive (EC50 $\approx$ 10 µg/L) to some chelators which are resistant to secondary treatment (e.g., EDTA, DTPA). As new studies are conducted, this concern or question can be incorporated and the risk can be evaluated.

6.2 SUBORGANISMAL, PHYSIOLOGICAL OR BIOMARKER RESPONSES TO PULP MILL EFFLUENTS

A variety of suborganismal, physiological or biomarker responses to pulp mill effluents have been measured in a number of tissues and cells. These tissues and cells have come from field-collected organisms as well as from organisms that have been exposed under carefully controlled conditions in mesocosms/microcosms or in laboratory exposures. Both responses indicative of exposure and responses indicative of effects have been measured. Presented below is a brief summary treatment of these data since 1994.

6.2.1 Measures of exposure

Enzyme induction has been used as a reliable measure of exposure. Mixed-function oxygenases (MFOs) are multi-enzyme complexes based on cytochrome P-450 that catalyze oxidation of various low molecular weight substrates. MFO is inducible by a variety of compounds that can bind to the Ah receptor. MFO activity is often determined from the rate of reaction of substrates with liver microsomes based on the reaction of EROD. Thus, EROD activity measurements are indicative of MFO induction and MFO induction is indicative of exposure to substances with the capacity to induce enzyme activity. The absence of significant MFO induction is good evidence of the absence of inducers at levels expected to cause adverse effects. However, significant MFO induction in exposed organisms is indicative of exposure and the potential for adverse effects if the inducers are identified and the strength of their interaction with the AhR is determined. Pulping liquors, both weak black liquor from kraft pulping and spent liquor from alcohol pulping, are potent MFO inducers. Products of resin acids are also potent MFO inducers. Historically, bleaching has also been a source of inducers. However, it is not clear that any portion of the pulp and paper process (species pulped, operation, bleaching etc.) has as great an effect on MFO induction as the efficacy of secondary treatment systems in reducing induction potential of effluents (Martel and Kovacs, 1996).

Other markers of exposure have included tissue levels of chlorophenolic compounds (Owens *et al.*, 1994), recombinant receptor/reporter gene assays (Zacharewski *et al.*, 1996, and P4501A protein antibodies (Owens and Lehtinen, 1996).

6.2.2 Measures of response

Several parameters have been used to predict reproductive responses of organisms in pulp and paper mill effluents. Levels of steroid hormones have considerable promise for prediction of impacts on sexual maturity and fecundity. Life cycle studies showed a good correlation between depressed circulatory steroid levels and altered secondary sexual characteristics with changes similar to those observed in wild fish (Robinson *et al.,* in press). However, the mechanistic links between reproductive effects and a chemical cause are weak. Thus, no process modifications or effluent treatment strategy can be recommended to eliminate them. Plant sterol hormones such as β-sitosterol can depress circulating steroid levels (Tana *et al*., 1994) and increased vitellogenin production.

6.3 BIOACCUMULATION

Recent changes in process and wastewater treatment have led to dramatic decreases in TCDDs and TCDFs in fish in receiving systems (Swanson *et al*., 1996). This decline in TCDDs and TCDFs in fish tissue has been observed at a number of locations after the bleaching process was changed. In fact, Sonnenberg *et al*. (1994) suggested that much of the difference in bioaccumulated chlorinated substances between upstream and downstream fish was due to the fact that fish downstream from pulp mills are typically fatter (contain more lipids) than upstream fish and that the bleaching process may not be the most important source of organochlorines in fish downstream from pulp mills.

6.4 TEMPORAL TRENDS IN BIOLOGICAL RESPONSES TO PULP MILL EFFLUENTS.

There are several obvious temporal trends in biological responses to pulp mill effluents. Clearly, these are related to alterations in exposure as a result of changes in process, raw material use, and effluent treatment. A general trend potentially influencing exposure has been rapid conversion to elemental-chlorine free (ECF) bleaching sequence during the past few years (Deardorff *et al.*, 1995). This has led to notable decreases in lipophilic chlorinated substances (e.g., PCDD/PCDFs) in effluents. Further, recognition of the contribution of well-managed biological or secondary treatment systems to reduction in concentrations and effects of polychlorinated phenolic compounds has led to additional gains in terms of exposures. Consequently, fish tissue samples have shown dramatic declines in dioxin concentrations with concomitant lifting of consumption advisories.

In general, responses of communities and populations to improvements in waste treatment and pulping processes have been positive (Munkittrick *et al.*, 1992; Axegård *et al.*, 1993, Deardorff *et al.*, 1995, Carey *et al.*, 1996). In a few cases, there are still apparently issues to be resolved. In other cases, the improvements have had negligible effects since there were no impacts associated with the previous operating conditions (Deardorff *et al.*, 1995). In other cases, the necessary process changes required are not known at present, but secondary wastewater treatment and 100% ClO_2 substitution were not sufficient to alleviate reproductive responses in fish. Tight controls of process streams that can constitute sources of bioactive compounds including spills and black liquor losses as well as recovery of foul condensates may contribute to resolution of the few cases where effects are still observed (Axegård *et al.*, 1993).

6.5 IMPLICATIONS FOR CONTINUING ENVIRONMENTAL MANAGEMENT OF PULP MILL EFFLUENTS.

Any strategy employed to foster reductions in environmental risks should be evaluated on a holistic basis (e.g., life cycle analysis, risk assessment). For example, we do not need to trade an aquatic problem for an atmospheric one. Similarly, we do not need to spend exorbitant amounts of money correcting a “problem” that does not exist.

Before risk assessments of pulp-mill effluent receiving waters are conducted it is crucial to understand the type of target ecosystem and the functional principles of the particular effluent receiving water body. For example, the functional mechanisms of rivers are different from those of lakes and coastal ecosystems. The functional characteristics of rivers are largely based on imported energy, whereas the functional characteristics of lakes and litoral costal systems are based on their own autotrophic capacity. Autotrophic capacity is, in turn, a function of nutrient input and light energy (latitude, Lehtinen *et al.*, (1996). Receiving waters at different latitudes may therefore show profoundly different capacities to assimilate loads of anthropogenic substances. Such factors have rarely been taken into consideration in risk assessments. Autotrophic production in boreal regions, during which there is net oxygen evolution and carbon dioxide fixation, is restricted to a short period, 2-3 months in the year, whereas ecosystems in more equatorial latitudes may function autotrophically all year round. Such functional differences are likely to modify the effects of effluents. Therefore, risk assessments of pulp mill effluents should, in a case-by-case fashion, consider ecosystem function as a key property.

The effects of long-term exposure to pulp and paper mill effluents are deserving of continued evaluation because of their high volume introduction into receiving systems. This recommendation is made since our evaluation "tools" and "strategies" are constantly evolving and improving. However, any decisions made based upon this monitoring must be made with appropriate consideration of the ecological relevance of the data. Long-term and continuous exposure to effluents may cause changes in reproductive cycles, reduced or enhanced growth, changes in the age-structure of populations, or inhibit an organism's ability to cope with stress. However, all of these effects are not necessarily adverse unless they have been evaluated in the light of their environmental significance.

Any risk mitigation strategies employed should be evaluated on a holistic basis (e.g., life cycle analysis, risk assessment). For example, we do not need to trade an aquatic problem for an atmospheric one. Similarly, we do not need to utilize scarce resources correcting a "problem" that does not exist. The effects of long-term exposure to pulp and paper mill effluents are deserving of continued evaluation because of their high volume introduction into receiving systems. However, any decisions made based upon this monitoring must be made clear understanding of the ecological relevance of the data..

7 UNCERTAINTIES

In reviewing the scientific information for this report, a number of uncertainties and attendant needs were identified. These are quite general and address the following:

> More information is needed on the types of substances produced in ECF and TCF bleaching processes. These needs are particularly directed at non-chlorinated substances and their biological activity.
>
> Future studies of the environmental consequences of modifications to pulp mill technology must be made in a much more systematic manner so that the biological implications of these changes can be assessed in the absence of experimental confounding.
>
> Future monitoring of the effects of pulp mill effluents on organisms in the receiving environment must be made with a clearer understanding of the ecological significance of these changes.

More efficient monitoring of the influences of changes in raw materials, the process technology used, and wastewater treatment is required.

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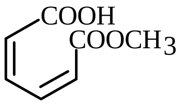
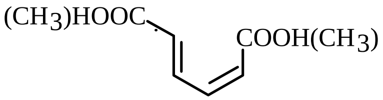
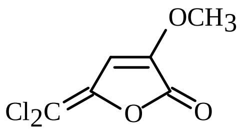
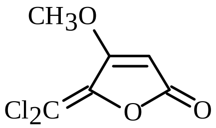
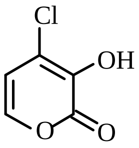
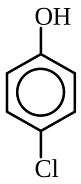
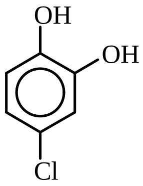
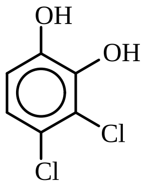
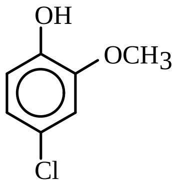
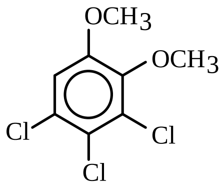
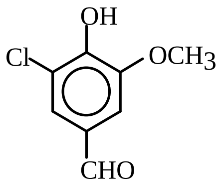
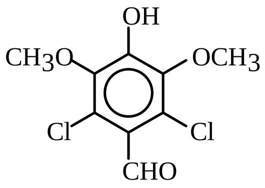
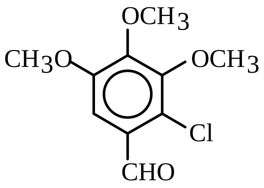
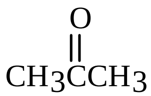
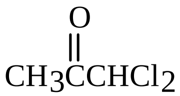
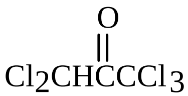
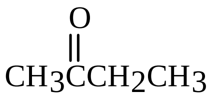
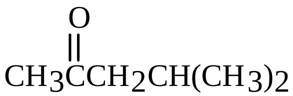
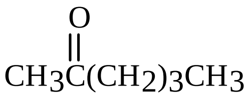
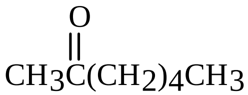
APPENDIX 1 COMPOUNDS IDENTIFIED SINCE 1993 IN EFFLUENT FROM MILLS EMPLOYING ECF BLEACHING

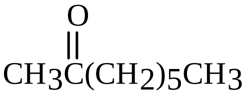
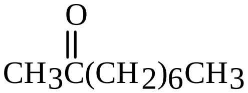
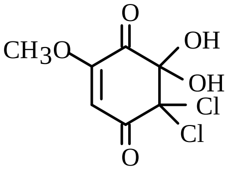
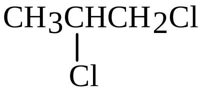
Compounds listed in this Appendix were first reported in the literature subsequent to the previous review and risk assessment in 1993 by Solomon *et al*. Most of the compounds are either non-chlorinated or have low degrees of chlorination and are expected to be readily biodegradable. Many of the compounds were detected in studies of volatiles originating in the bleach plant (Juuti *et al*., 1996; NCASI, 1994b) and have chemical structures which suggest they may originate from sources other than pulp. The chlorocymenes, chloronaphthalenes and chlororetenes were reported in final mill effluent after biological treatment. Since bleaching effluent from these mills was not analysed, it is not known whether these compounds were formed during bleaching or whether they originate from historical deposits in lagoon sediment (see Section 2.4).

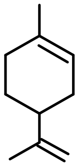
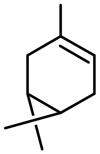
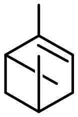
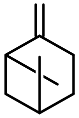
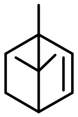
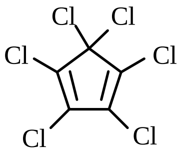
Compound	Chemical Structure	Identified in	
		Bleaching Effluent	Final Mill Effluent
(2Z,4Z)-Hexadienedioic acid monomethyl ester		Vilen *et al.*, 1996	-[1]
(2E,4Z)-Hexadienedioic acid monomethyl ester		"	-
Chloroacetic acid methyl ester	$ClCH_2COOCH_3$	Juuti *et al.*, 1996	ND[2]
Dichloroacetic acid methyl ester	$Cl_2CHCOOCH_3$	"	ND
3-Methoxy-5-dichloro-methylene-2(5H)-furanone		McKague and Grey, 1996	-
4-Methoxy-5-dichloro-methylene-2(5H)-furanone		"	-
4-Chloro-3-hydroxy-2H-pyran-2-one		Smith *et al.*, 1994a	-

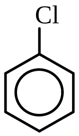
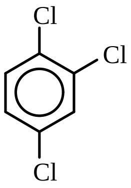
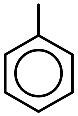
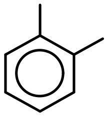
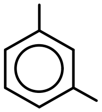
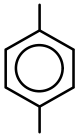
Compound	Chemical Structure	Identified in	
		Bleaching Effluent	**Final Mill Effluent**
4-Chlorophenol		NCASI, 1994a	ND
4-Chlorocatechol		Smith *et al.*, 1995 NCASI, 1994a	ND
3,4-Dichlorocatechol		Smith *et al.*, 1995 NCASI, 1994a Pryke *et al.*, 1994	ND
4-Chloroguaiacol		Pryke *et al.*, 1994 NCASI, 1994a	ND
3,4,5-Trichloroveratrole		ND	Pryke *et al.*, 1994

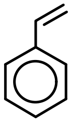
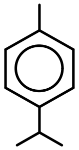
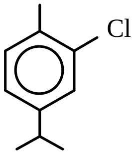
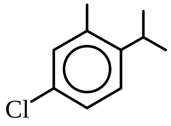
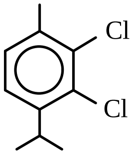
Compound	Chemical Structure	Identified in	
		Bleaching Effluent	**Final Mill Effluent**
Acetaldehyde	CH_3CHO	NCASI, 1994b	-
Pentanal	$CH_3(CH_2)_3CHO$	Juuti *et al.*, 1996	-
Hexanal	$CH_3(CH_2)_4CHO$	"	-
Heptanal	$CH_3(CH_2)_5CHO$	"	-
Octanal	$CH_3(CH_2)_6CHO$	"	-
Nonanal	$CH_3(CH_2)_7CHO$	"	-
5-Chlorovanillin		NCASI, 1994a	ND
Dichlorosyringaldehyde		Smith *et al.*, 1995	-
Chloro-3,4,5-trimethoxy-benzaldehyde		"	-

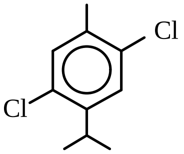
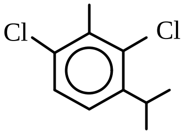
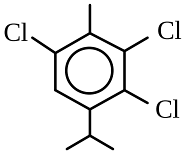
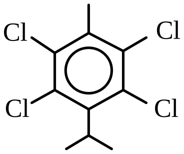
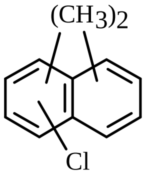
Compound	Chemical Structure	Identified in	
		Bleaching Effluent	**Final Mill Effluent**
Acetone	 $CH_3\overset{\overset{O}{\|}}{C}CH_3$	Kincaid, 1994 Thut and Persinger, 1994 Wiesemann, 1994	-
1,1-Dichloroacetone	 $CH_3\overset{\overset{O}{\|}}{C}CHCl_2$	Juuti *et al.*, 1996	-
Pentachloroacetone	 $Cl_2CH\overset{\overset{O}{\|}}{C}CCl_3$	Smith *et al.*, 1994b	-
2-Butanone	 $CH_3\overset{\overset{O}{\|}}{C}CH_2CH_3$	Kincaid, 1994 Thut and Persinger, 1994 Wiesemann, 1994	-
2-Pentanone	 $CH_3\overset{\overset{O}{\|}}{C}CH_2CH_2CH_3$	Juuti *et al.*, 1996	-
4-Methyl-2-pentanone	 $CH_3\overset{\overset{O}{\|}}{C}CH_2CH(CH_3)_2$	NCASI, 1994b	-
2-Hexanone	 $CH_3\overset{\overset{O}{\|}}{C}(CH_2)_3CH_3$	Juuti *et al.*, 1996	-
2-Heptanone	 $CH_3\overset{\overset{O}{\|}}{C}(CH_2)_4CH_3$	"	-

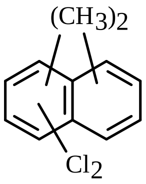
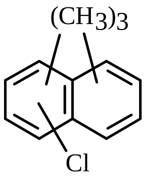
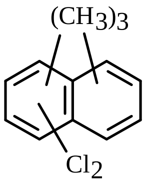
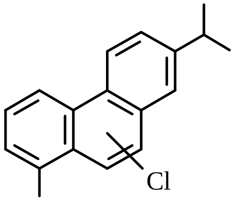
Compound	Chemical Structure	Identified in	
		Bleaching Effluent	**Final Mill Effluent**
2-Octanone	 $CH_3\overset{O}{\overset{\|}{C}}(CH_2)_5CH_3$	"	-
2-Nonanone	 $CH_3\overset{O}{\overset{\|}{C}}(CH_2)_6CH_3$	Juuti *et al.*, 1996	-
5,5-Dichloro-6,6-dihydroxy-2-methoxy-2-cyclohexene-1,4-dione		McKague and Grey, 1996	-
Dichloromethane	CH_2Cl_2	NCASI, 1994b	-
Carbon tetrachloride	CCl_4	"	-
Bromodichloromethane	$CHCl_2Br$	Juuti *et al.*, 1996	-
1,2-Dichloroethene	ClCH=CHCl	NCASI, 1994b	-
1,2-Dichloropropane	 $CH_3\underset{Cl}{\underset{\|}{C}}HCH_2Cl$	Juuti *et al.*, 1996	-
Hexane	$CH_3(CH_2)_4CH_3$	NCASI, 1994b	-

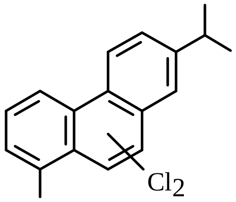
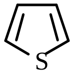
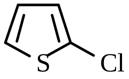
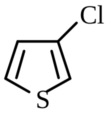
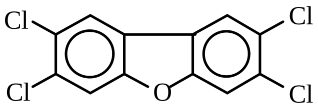
Compound	**Chemical Structure**	**Identified in**	
		Bleaching Effluent	**Final Mill Effluent**
Limonene		Juuti *et al.*, 1996	-
3-Carene		Juuti *et al.*, 1996	-
α-Pinene		NCASI, 1994b	-
β-Pinene		"	-
Camphene		Juuti *et al.*, 1996	-
Hexachlorocyclopentadiene		"	-

Compound	**Chemical Structure**	**Identified in**	
		Bleaching Effluent	**Final Mill Effluent**
Benzene		NCASI, 1994b	-
Chlorobenzene		NCASI, 1994b	-
1,2,4-Trichlorobenzene		"	-
Toluene		"	-
o-Xylene		"	-
m- & *p*-Xylenes	 	"	-

Compound	**Chemical Structure**	**Identified in**	
		Bleaching Effluent	**Final Mill Effluent**
Styrene		"	-
p-Cymene		Juuti *et al.*, 1996	-
2-Chloro-*p*-cymene		-	Rantio, 1995
5-Chloro-*o*-cymene		-	"
2,3-Dichloro-*p*-cymene		-	"

Compound	Chemical Structure	Identified in	
		Bleaching Effluent	Final Mill Effluent
2,5-Dichloro-*p*-cymene		-	"
2,6-Dichloro-*m*-cymene		-	"
2,3,6-Trichloro-*p*-cymene		-	Rantio,1995
Tetrachloro-*p*-cymene		-	"
Chlorodimethyl- naphthalenes		-	Koistinen *et al.*, 1994

Compound	Chemical Structure	Identified in	
		Bleaching Effluent	Final Mill Effluent
Dichlorodimethyl-naphthalenes		-	"
Chlorotrimethyl-naphthalenes		-	"
Dichlorotrimethyl-naphthalenes		-	Koistinen *et al.*, 1994
Chlororetenes		-	"

Compound	Chemical Structure	Identified in	
		Bleaching Effluent	**Final Mill Effluent**
Dichlororetenes		-	"
Thiophene		Juuti *et al.*, 1996	-
2-Chlorothiophene		"	-
3-Chlorothiophene		"	-
2,3,7,8-Tetrachloro-dibenzofuran		Stinchfield and Woods, 1994 Wiesemann, 1994	-
Dimethyl sulfide	CH_3SCH_3	Juuti *et al.*, 1996	-
Dimethyl disulfide	CH_3SSCH_3	NCASI, 1994b	-
1,1,3-Trichlorodimethyl sulfone	$ClCH_2SO_2CHCl_2$	O'Connor *et al.*, 1994 Smith *et al.*, 1994b	-
1,1,3,3-Tetrachloro-dimethyl sulfone	$Cl_2CHSO_2CHCl_2$	Smith *et al.*, 1994b	-

Compound	Chemical Structure	Identified in	
		Bleaching Effluent	Final Mill Effluent
Trichloronitromethane	Cl_3CNO_3	Juuti *et al.*, 1996	-

1. Not analysed.
2. Not detectable.

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