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Toxicity Assays in the Pulp and Paper Industry — A Review and Analysis

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Abstract

This paper endeavors to summarize and clarify knowldege regarding toxicity assays utilized in the pulp and paper industry. A wide variety of toxicity assays are described and evaluated including those based on: *Ceriodaphnia*, fathead minnow, salmonids and trout, Microtox, *Nitzschia closterium*, Ames *Salmonella* Assay, and enzyme induction including MFO and EROD.

Standard toxicological terminology and definitions are discussed. Also included is a discussion of relationships between toxicity and effluent parameters, including chlorophenols, chlorinated dioxins and furans, and extractives.

Introduction

The field of toxicity testing is complicated by lack of agreement between experts in the field and regulatory agencies. The result is that there are many complex protocols with few objective criteria to facilitate a preference for the use of one protocol over another(1). Another difficulty is that terminology is extensive, highly specialized, and varies from one research group to another(1). This paper endeavors to summarize and clarify knowledge of toxicity assays currently utilized in the pulp and paper industry.

Definition of Terms

The terms "acute" and "chronic" are often utilized ambiguously to describe toxicity. Both can refer to either the length of exposure or lenght of response. In general, acute toxicity implies effects having a sudden onset and lasting a short time. When referring to a stimulus, acute means the stimulus is severe enough to induce a response rapidly. The duration of an acute aquatic toxicity test is generally 4 days or less and mortality is the response measured(2).

A chronic stimulus lingers or continues for a long time. The term often signifies periods from several weeks to years, depending on the reproductive life cycle of the aquatic species in question. Chronic exposure typically induces response with slow progress and long continuance(2). Often the terms chronic and sublethal are used interchangeably. It is important to understand that lethal or nonlethal responses can occur either acutely (quickly) or chronically (longer response time).

A life cycle study is one in which all the significant life stages of an organism are exposed to the test material(2). Usually, this involves an entire reproductive cycle of the test organism.

The definitions of typical toxicity assay endpoints also need clarification. The median lethal concentration, LC50, is the concentration that results in death for 50% of the test organisms. Likewise, the median inhibition concentration, IC50, is the concentration that reduces the fecundity of the test organism by 50%. The median effective concentration, EC50, is the concentration that induces a sublethal response of 50% in the test organism. If an EC50 is cited, a statement of the sublethal response needs to be clearly defined. LC50, IC50, and EC50 are time-dependent values. Therefore, the test duration (e.g., 24-hr or 96-hr) that yielded the observed values must be stated.

The lowest observed effect concentration (LOEC) is the lowest concentration of the test material used in the toxicity assay that has a statistically significant adverse effect on the test organism compared with the control. Likewise, the no observed effect concentration (NOEC) is the highest concentration of a test material, used in the assay, which has no statistically significant adverse effect on the exposed test organism compared with the control.

If an assay is referred to as *in vivo*, it is an assay occurring within an intact animal or organism. This is common for aquatic assays. On the other hand, if the assay is *in vitro*, it is outside the intact organism. This term is generally applied to experiments involving biochemical reactions in tissue fragments or fractions.

Measurements of Toxicity

Because there are many possible toxic responses, it is difficult to detect and measure all of them. One test method, or even a battery of test methods, can not evaluate all possible responses. Often a particular bioassay will be weak at determining toxicants that are in a particular class of compounds. By using a battery of assays, there is a higher probability of detecting a potential toxin than by using only one test. However, when the number of bioassays is increased, there is also an increase in capital equipment, consumable supplies, and required personnel. In the long run, a balance must be found between the cost of a number of tests and the potential increase in information gathered(3).

Ceriodaphnia

Use of *Ceriodaphnia dubia* as a test species is the most common method for evaluating toxicity in the pulp and paper industry. *Ceriodaphnia*, a water flea, is an invertebrate.

The 7-day test method consists of exposing early life stage organisms to various concentrations of mill effluents. The mill effluent is diluted with either preconditioned or natural water which may need to be enriched. Dietary needs are met with trout chow, yeast, and Cerophyll (a cereal leaf product), which is administered regularly to the test species(4). *Ceriodaphnia* survival and reproduction rates are calculated and compared at five effluent concentrations(5). The neonate (baby) should be able to develop to sexual maturity and produce three broods of offspring during the 7-day test period.

It is generally accepted that reproduction is a more sensitive endpoint for toxicity evaluation than mortality(6). Reproduction is more sensitive than mortality by at least a factor of five. Reductions in fecundity are indicative of a chronic response, whereas mortality can be either an acute or chronic response.

Advantages of the *Ceriodaphnia* test method include completion of an entire life cycle evaluation during a relatively short (7-day) test period. Although the paper industry utilizes a 7-day test, the *Ceriodaphnia*'s average life span is 21 days. When compared to other organisms, such as the fathead minnow, *Ceriodaphnia* has been determined to be the most sensitive species(5). Another advantage is that a specific protocol has been developed by the EPA. Other environmental regulatory agencies and technical associations have also drafted procedures for *Ceriodaphnia* toxicity determinations(7).

One major difficulty in utilizing the *Ceriodaphnia* method is the time and cost commitment. The extensive time commitment and cost constraints limit the sample size as well as the number of concentrations that can be tested(8). Labor requirements are high; approximately 90 hours of labor are needed over a 7-day test period. Culture maintenance and expenses are additional(9).

Other problems involve the lack of understanding of *Ceriodaphnia* health and survival requirements. The nutritional needs are not well understood, and therefore, could be leading to death or disease(4). Water quality parameters, such as pH, hardness, alkalinity, and conductivity are not understood with regard to the health of *Ceriodaphnia*. There have been cases where the ambient water toxicity was reduced by the addition of effluents(9). One cause of "toxic" water may simply be lack of nutrients.

Other concerns include maintenance of *Ceriodaphnia* populations in the laboratory. Although *Ceriodaphnia* are relatively easy to culture, population fluctuations are frequent, and occasional crashes (death of all control organisms) occur(4, 9). In using the 7-day test, neonates must be between 2 and 24 hours old and all test specimens must be within four hours age of each other. One major difficulty arises in gathering sufficient numbers of neonates for testing. For this reason, as well as the fragility of the organisms, suggestions have been made to increase the age of the neonates to 24-48 hours old(9).

Little work has been done in the area of reproducibility and applicability of the *Ceriodaphnia* method, and demand is growing for further research in this area. In May 1990, The National Council of the Paper Industry for Air and Stream Improvement (NCASI) published a report on the intra- and inter-laboratory reproducibility of the *Ceriodaphnia* method(10). Results showed that "about half (56 percent) of the laboratories participating in the study were able to routinely complete the test successfully". The laboratories that participated were from industry, contractors, EPA, and universities that routinely conducted these tests. Several test specimens were provided to the labs and the five concentrations to be tested were specified. Even with identical test specimens and concentrations, the interlaboratory coefficients of variation are extremely high as can be seen in Table 1. Although the NCASI report investigated inter- and intralaboratory reproducibility, only interlaboratory results are cited here since many labs investigated were unable to successfully repeat the experiments.

Test Material	CV (%)* Lethality (LC50)	CV (%)* Reproduction (IC50)
NaCl #1	6.7	46.4
NaCl #2	61.9	65.0
potassium dichromate #1	70.8	30.8
potassium dichromate #2	38.2	48.9
utility effluent #1	56.4	57.7
utility effluent #2	0.0	27.3
pulp and paper effluent #1	0.0	28.3
pulp and paper effluent #2	12.0	7.5
overall mean	30.8	39.0

* all < or > values considered as actual values

Table 1. Interlaboratory coefficients of variation for seven-day lethality (LC50) and reproduction (IC50) with *Ceriodaphnia* toxicity assay(10).

For comparison purposes, inter- and intra-laboratory reproducibility have also been compiled for common analytical test methods. These are shown in Table 2.

EPA method	Intralaboratory CV range (%)	Interlaboratory CV range (%)
601 purgeable halocarbons by purge and trap	5-28	13-58
602 purgeable aromatics	7-18	15-71
604 phenols	15-37	20-45
605 benzidines	24-40	38-69
606 phthalate esters	1-80	12-73
607 nitroamines	13-36	21-46
608 organochlorine pesticides and PCBs	10-33	12-45
609 Nitroaromatics and Isophorone	13-45	26-60
610 PNAs	11-50	16-91
611 Haloethers	15-31	32-53
612 Chlorinated Hydrocarbons	16-24	26-41

Table 2. Inter- and Intralaboratory coefficients of variation for various EPA methods(10).

***Pimephales promelas* (Fathead Minnow)**

This 7-day method relies on measurements of growth and survival of newly hatched larval minnows. The method can be used to study several concentrations and effluents, but unlike the *Ceriodaphnia* method, the fathead minnow's weight is also monitored as a measure of growth.

The fathead minnow has the same advantages as the short 7-day *Ceriodaphnia* test method and a protocol has been established by the EPA. Unfortunately, using the fathead minnow assay also has several disadvantages.

According to the EPA protocol, the fathead minnow is fed brine shrimp. This poses two problems. First, newly hatched fry may be unable to eat large shrimp for several days. Secondly, since fathead minnows are sight-feeders, color effects of effluents may reduce their ability to locate food. Ambient laboratory lighting may also limit sight-feeding(4, 9).

Concern also surrounds the young age of the organism. Due to the fragility of the newly hatched fry during the first days of life, being placed in a foreign solution may cause shock, damage, or death from even the safest of effluent samples. The delicacy of these small, fragile organisms can lead to damage during handling prior to and during the study.

Like *Ceriodaphnia*, the fathead minnow's dietary, water quality, and biological requirements are uncertain. These areas of uncertainty must be explored further in order to ensure accurate results in testing for chronic toxicity.

Salmonids and Trout

Salmonids, trout, and other species indigenous to pulp and paper mill outflow areas have been used for chronic toxicity testing. Trout and salmonids are monitored for growth changes (both length and weight) as well as survival and reproduction.

Advantages include using an organism higher in the food chain. These organisms are also more likely to be found directly adjacent to effluent release sites, thus giving a more accurate representation of chronic dangers to the aquatic environment.

The lengthy testing period (8 months to 2 years) is most often the factor discouraging use of these methods. The testing period is based on the life cycle of the organism; a salmon goes through a life cycle of approximately one year(11).

Storage and living areas required for the fish are extensive. As an example, Seim *et al.* used six wooden troughs 3.3 meters long, 66 cm wide, and 25 cm deep. Equipment needed to support the study were one 800 gallon tank, two refrigerated 350 gallon and several 500 gallon tanks(12). When using salmonids and trout for toxicity evaluations, capital investment is relatively large in comparison with other methods.

When dealing with more complex organisms, variability of results becomes higher. Younger fish are fairly uniform in size and weight, resulting in lower levels of variability. But as organisms age, variability, unrelated to the effluent being tested, increases(5).

As in the *Ceriodaphnia* and fathead minnow methods, there are several analytical procedural problems with utilizing Salmonids and trout. For the first three weeks of life, trout retain a yolk sac. For thorough chronic toxicity testing, the parent would need to be exposed to the effluent before and during spawning in order for offspring to be exposed for a full lifetime. Salmonids are also sight feeders. Growth may be minimized by the effluent's inhibition of the salmonid's ability to seek nourishment.

James River Corporation has published extensively on chronic toxicity testing methods and results. They have determined water fleas and fathead minnows more "appropriate, acceptable, and logically defensible" than salmonids for chronic toxicity testing(5).

Respiration Rate

Oxygen consumption is a useful measure of sublethal toxicity effects since energy processes are indicators of overall physiological health(2). Toxic agents damage respiratory membranes of aquatic animals. Asphyxiations can be brought on by toxicants reacting with some constituent of the mucus secreted by fish gills. Exposure to a toxicant may also result in gill tissue deterioration including swelling of epithelial cells, adhesion of secondary lamellae, detachment of epithelium from the pillar cell system, discharge of mucous cells, and hematomas. Monitoring respiration may be justifiable for pollutants that greatly affect respiratory membranes or respiratory processes, but organochlorines do not appear to be in this category. However, trace metals do result in respiratory damage(2).

Microtox

The Microtox™ assay, marketed by Microbics Incorporated, indirectly monitors the metabolic response of an ocean-dwelling bacterium to determine toxicity. *Vibrio fisheri lux* (previously referred to in the literature as *Photobacterium phosphoreum*) naturally emits visible light as a result of metabolic reactions

which liberate energy(13). When exposed to toxic compounds, the enzymes which promote this reaction are inhibited; the resulting decrease in light output is directly proportional to the toxicity of the sample. Light reduction is the direct measurement; EC50 is the concentration of the test sample which results in a 50% reduction in light output.

Many researchers have evaluated the comparative sensitivity of Microtox™ to that of other common aquatic assays, with conflicting conclusions. Qureshi *et al.* demonstrated that for organic compounds, pulp and paper effluents, and oil refinery effluents, Microtox™ sensitivity was comparable to *Salmo gairdneri* (rainbow trout), *Spirillum volutans* (a bacterium), and *Daphnia magna* (a crustacean) bioassays(14). Microtox™, in general, was found to be the most sensitive to industrial effluents; in some cases, Microtox™ was substantially more sensitive.

Firth and Backman found Microtox™ to have good correlations with acute rainbow trout LC50 values and *Ceriodaphnia* chronic toxicity values for both treated and untreated pulp and paper mill wastewaters(8, 15). Microtox™ values were more sensitive than rainbow trout mortality but less sensitive than *Ceriodaphnia* reproduction. However, in another study, Microtox™ was found to be more sensitive than the *daphnid* chronic assay(16). Fraser completed a correlation study comparing Microtox™ with Rainbow trout and *Daphnia magna*. Results indicate that Microtox™ results correlate well with rainbow trout, but not with *Daphnia magna*(17). Stauber *et al.* compared the Microtox™ toxicity of bleached eucalypt kraft mill effluents to a phytoplankton (*Nitzschia closterium*) growth inhibition test. The effluents exhibited greater toxicity to *Nitzschia* (1-20x more toxic) than Microtox™(18, 19).

When Renberg evaluated data from both mill and laboratory experiments, no correlation was found between Microtox™ toxicity and *Ceriodaphnia* or algal toxicity. On the other hand, Renberg did find that when the use of Microtox™ was limited to samples from similar sources, such as pulp mill effluents, the response agreed with that of *Ceriodaphnia*(20). Springer and Bazarow evaluated compounds typically found in mill effluents, but found no significant correlation between Microtox™ and rainbow trout, fathead minnow, *daphnia magna*, or *selenastrum capricornutum*(21).

Microtox™ has been shown to exhibit satisfactory sensitivity to pulp and paper process effluents and is a relatively quick, easy, and economical method for evaluating toxicity. No culture maintenance is necessary since the bacteria arrive freeze-dried and remain frozen until immediately prior to use. Another benefit of the Microtox™ assay is the small sample volume required for testing.

The EC50 is determined by the decrease in luminescence of bacteria after exposure to the effluent in question. This test method gives no information or indication as to what, if any, specific effects the effluent may impose on marine life.

Nitzschia closterium

Nitzschia closterium is a unicellular marine diatom (colonial algae) which is widely distributed in Australian waters. Algal species have been widely used for toxicity assays; however, this species is given attention in this paper because there is currently a strong movement in Australia to begin toxicity testing with native organisms to evaluate kraft effluents. The *Nitzschia* cells divide 1.4 times per day so that a number of generations of cells are exposed to the effluent over the 3-day bioassay(18). This allows for a short-term, sublethal, chronic bioassay.

When *Nitzschia* EC50 (72 hours; reduction of cell division rate) was compared to Microtox 15-min EC50, the effluent was found to be 1-20x more toxic to *Nitzschia* than to Microtox(18). The *Nitzschia* assay has also been compared to other Australian native organisms, such as *Hormosira banksii* (brown macroalgal fertilization test) and Tasmanian blenny (survival of larval stages of a tidepool fish), and the *Nitzschia* was the most sensitive and the most reproducible(19). This assay needs further development, but appears to have much promise in providing Australian researchers with a native toxicity assay.

Ames *Salmonella* Test

The Ames test, developed by Ames in 1971, is the best known and accepted method for determining mutagenic potential and is accepted around the world as a standard method(22). It is based on specially prepared mutants of the bacterium *Salmonella typhimurium*. As a result of treatment, the bacteria have lost the ability to synthesize histidine, an amino acid. The bacteria can not grow unless histidine is added to the culture medium. When exposed to a mutagenic compound, the introduced damage to the histidine mechanism can be repaired by mutation and the bacteria will regain the ability to grow on a histidine-free medium(23).

Numerous strains of *Salmonella typhimurium* have been isolated in order to detect a multitude of mutation mechanisms. Many of the strains have been used by different researchers investigating kraft bleaching effluents. For pulp bleaching effluents, the strain that has been found to be the most sensitive for detecting mutagens is TA 100(24).

In order to simulate the conditions in a mammalian body, rat liver microsomes (S9 fraction), which contain the enzymes responsible for the most important metabolism of chemicals in the mammalian body, can be added to the bacterial cultures. Most of the known substances which give rise to cancer, such as vinyl chloride, aflatoxin, benzopyrene, and benidine, require a metabolic change in order to become active carcinogens(25). However, chlorination stage effluents have been found to have a reduction in mutagenic potential when liver microsomes are present, indicating a metabolic detoxification of the mutagens(25).

It has been determined that spent chlorination liquors of most pulp types exhibit mutagenic activity when tested according to the Ames test(24, 26). The effect is apparently caused by various chlorinated compounds with relatively low molecular masses(27). The bacterial mutagenicity of chlorinated effluents was initially reported in Sweden in 1977, and has since been confirmed by workers at the Pulp and Paper Research Institute of Canada (PPRIC) and elsewhere(28). A majority of the total mutagenic activity, estimated to be 75%, has been found to originate from the lipid-soluble fraction(24, 27, 29). In spent chlorination liquors, approximately 85% of the mutagenicity has been found in the fraction containing compounds with low molecular weights(23, 30). Chlorination liquors tested for mutagenicity with the Ames test have shown the number of mutant revertants to be linearly related to the chlorine ratio(31). Hypochlorite, extraction, and chlorine dioxide bleaching effluents have been found to have little or no mutagenic activity(25). It has been estimated that oxygen prebleaching may reduce the total mutagenicity of a subsequent CD stage effluent by 50%(23). A linear relationship exists between the number of mutants and the chlorine dioxide/chlorine ratio, the number of mutants decreasing with increasing chlorine dioxide/chlorine ratio. With pure chlorine dioxide, the number of mutants is close to the number found in the control sample(23).

Enzyme Induction

Enzymes are biological catalysts, substances that increase biological reactions without being changed themselves in the overall process. When a toxic compound is present, three types of observations are possible in enzyme systems. First, enzymatic production may not be affected. Secondly, enzymatic production may be increased to aid detoxification reactions. Thirdly, enzymatic production may be inhibited by the toxicant and results in a reduction of enzyme available for necessary biological reactions.

Many toxicity assays have used enzyme monitoring to evaluate the toxicity of a compound or solution. The following invertebrates have been monitored for an induction of mixed-function oxygenase (MFO) enzymes: *Nereis virens* (sandworm), *Uca pugilator* (fiddler crab), *Mytilus galloprovincialis* (mussel), and *Collinectes sapidus* (blue crab). Vertebrates have also been used for this purpose, such as: *Fundulus heteroclitus* (mummichog), *Cittaricthys sordidus* (pacific sanddab), *Leuciscus cephalus* (chub), *Salmo gairdneri* (rainbow trout), *Barbus barbus* (barbel), *Chondrostoma nasus* (nase), and *Perca fluviatilis* (perch)(32).

When an organism such as a salmonid, trout, or even a human is exposed to a xenobiotic (toxicant), enzymes, primarily in the liver, react in order to remove the toxicant from the body(33). There are many enzyme systems available; however, the major system is the cytochrome P-450, otherwise known as the mixed-function oxygenase (MFO) system. The MFO enzymes are found in the phospholipid matrix of the endoplasmic reticulum(34).

In brief, the enzymes attempt to detoxify the zenobiotic by adding a hydroxyl moity which will result in increasing the water solubility of the toxicant allowing it to be excreted from the organism(35). When exposed to a zenobiotic, the enzyme system has the ability to increase its capacity to metabolize the toxicants(36). To determine toxicity, enzyme induction, or the increase in enzymes present in an organism exposed to a test material, can be compared to a control organism.

There are two substrates used for measuring monooxygenase activity: benzo(a)pyrene (i.e., arylhydrocarbon hydroxylase activity, AHH) and 7-ethoxyresorufin deoxyethylase (i.e., EROD activity). Since there is a strong linear correlation between AHH and EROD activity, only one assay, preferably EROD, is necessary(37).

Preliminary evidence indicates that the kraft process may be a source of MFO-inducing substances(38). However, MFO response patterns have been found not to reflect the distribution of AOX in the water(39).

MFO induction responses did parallel the gradients of phenolics in water and the levels of TCDD equivalents found in fish tissues(39). On other occasions, EROD induction was found not to correlate with either dioxins or furan body burdens(37). It should be emphasized that EROD induction is a response to a broad spectrum of hydrophobic compounds, so induction is not evidence of the presence of any particular class of compounds whether it be furans, dioxins, or other organochlorines. In fact, Birkholz and Swanson suggest that the compound(s) causing MFO induction are non-chlorinated since induction is seen in fish exposed to both bleached and unbleached effluents(36).

Researchers found that MFO increased for both bleached and unbleached kraft effluents over controls(38). The presence of oxygen delignification, high substitution, and secondary treatment did not eliminate the MFO response(38). Data show that the replacement of chlorine in the bleaching of kraft pulp by chlorine dioxide or nonchlorine-containing compounds such as peroxide does not significantly alter an effluent's ability to induce MFO activity(38). The use of chlorine for kraft pulp bleaching did not appear to be an important factor in determining the ability of final mill effluents to cause MFO enzyme induction in fish(40). New bleaching technologies with secondary treatment also did not eliminate the MFO response(38).

Effluent Parameters and Their Relationship to Toxicity

The impact of pulp mill discharges on receiving waters is dependent upon the oxygen demand, suspended solids, pH, color, and toxicity of the effluent. It is important to note that frequently it is impossible to segregate effects due to toxicity from those due to other effluent characteristics(41). Variables which affect effluent composition include: raw materials, woodyard operation, pulping processes, degree of closure and spill control, handling and use of condensates, process chemicals, and control of operations outside of the pulp stream(42). In natural waters, the impact of the effluent upon the receiving water is dependent upon not only effluent characteristics, but also the composition of the receiving water, relative flows, and conditions affecting dilution and dispersal of the wastes(43).

Toxicity relationships to groups of compounds are discussed below. However, it is important to preface this discussion by noting that additive and synergistic effects complicate the toxicity analysis of bleaching liquors. Assessing the risks posed by chemicals in industry is especially difficult, not only because of the large number of chemicals involved, but also because these chemicals often occur as complex mixtures of poorly known composition. The effluents from pulp and paper mills include dissolved lignin and cellulose degradation products, other wood extracts, and chlorinated organics. It is more

study the whole mixture rather than specific compounds since it is the entire mixture that is released into the environment(3).

AOX

AOX is a chemical analysis procedure which measures the total organically-bound chlorine, without reference to species(44). Researchers agree that AOX is not related to toxicity and that appropriate internal measures taken at kraft pulp mills combined with biological treatment of the effluents eliminate acute lethal toxicity and the remaining sub-acute toxicity that can be characterized as "weak toxic effects" are not caused by organochlorine substances, but rather by neutral, non-chlorinated compounds(45). O'Conner, *et al* conducted tests on a variety of bleached and unbleached kraft mill effluents, sampled before and after biological treatment systems, and showed that there was no correlation between AOX and acute or sub-lethal toxicity to *Ceriodaphnia* or fathead minnows(46). Figure 1 illustrates the lack of correlation between AOX and toxicity.

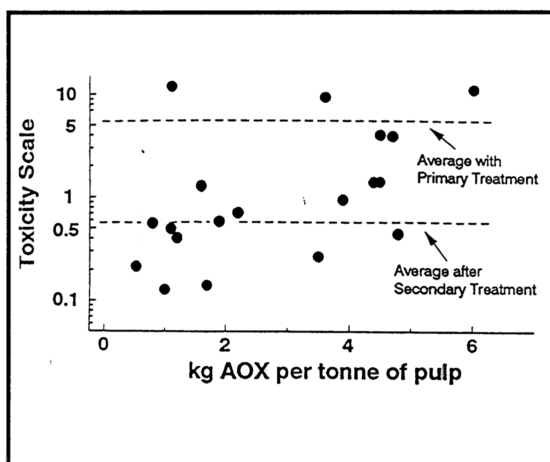

Figure 1. AOX vs. Chronic Ceriodaphnia Toxicity (after biological treatment) of Effluents from Bleached and Unbleached Pulping Facilities(47)

Lehtinen found when chlorine use was reduced or when effluents were more thoroughly treated, a different spectrum of chlorinated compounds was detected in the effluent and toxicity (mortality, growth, enzyme induction) decreased(39). One mill showed significant correlations between low (<1000) MW TOX concentrations and acute toxicity in rainbow trout and *Ceriodaphnia* chronic toxicity(48).

When a mill implements process changes to reduce AOX discharges, many other characteristics of the effluents also change, systematically reducing discharges of many pollutants(45). The reduction in total organics discharged, which accompanies implementation of AOX control measures, is probably the reason for the toxicity reduction observed when effluents from mills with differing AOX discharge rates are compared(45).

There is abundant evidence that AOX measurements are ineffective in characterizing the toxicity of pulp mill effluents or for use as a parameter indicitive of environmental impact(45, 49). An independent scientific advisory group, convened by Procter and Gamble with the assistance of NCASI determined that "available data do not support a conclusion that chlorinated organic compounds discharged from bleached pulp mills are largely responsible for the damage to the aquatic environment observed in Sweden"(50). Rather than AOX, other properties of effluents, such as chronic toxicity measured with appropriate aquatic test organisms, or composition with respect to specific, persistent, bioaccumulable, and potentially toxic substances are more relevant(49).

Chlorophenols

Salkinoja-Salonen, *et al* were unable to demonstrate a positive correlation between sample toxicity and concentration of specific chlorophenolic compounds(51). Information on structure-activity relationships demonstrates that the degree of chlorination is the major factor determining the toxicity of phenolic compounds rather than specific parent ring structure(44).

Chlorinated Dioxins and Furans

Lab exposures of fish to chlorinated dioxins and furans result in epithelial cell damage, embryo-larval mortality, a possibility of impaired regulation of reproductive hormones, and a strong induction of hepatic MFO enzymes(52-56). Since most mills in the U.S. have reached non-detect levels of both chlorinated dioxins and

reached non-detectable levels of both chlorinated dioxins and furans, this is becoming less of a toxicity issue. Attention must be paid to the bioacçumulation potential which could lead to contamination of the higher food chain.

Extractives

Since alkaline pulping conditions ensure maximum solubilization of extractives into the pulping liquor(57), wood resin accounts for approximately 10% of the COD in pulping liquors(58, 59). Certain resin acids are known to bioaccumulate and to be toxic to aquatic organisms(60-62). A positive relationship has been found between toxicity and the concentration of resin acids present(63). Lab exposure of fish to resin acids causes lysis of red blood cells, increased breakdown of hemoglobin to bilirubin, and overloading of the conjugation pathway(64).

Scandinavian studies also suggest that resin and fatty acids and perhaps plant sterols originating from wood extractives are important toxicologically(65-68). In fact, much of softwood pulp effluent toxicity has been attributed to the resin acids, particularly dehydroabietic acid(69).

Summary

Chemical and biological data specific to a mill are usually necessary to evaluate and to define the relationship between the operation of the mill and the health of its receiving ecosystem(42). However, there are numerous toxicity assays that can be used to evaluate the potential impact upon various aquatic populations and to determine potential sources of toxicity.

No single test system is adequate to ensure the detection of all toxic effects of a particular compound or of a complex effluent. There is also no single "most sensitive species." For this reason, batteries of tests are now frequently used. However, increasing the number of bioassays results in additional expense for capital equipment, consumable supplies, personnel, and testing. In the final decision, a balance must be gained between the cost of a battery of tests and the potential increase in information.

No correlation has been found between toxicity and BOD, AOX, or TOC(41, 43, 70, 71). The relative contribution of chlorinated organic waste to acute toxicity has not been isolated from the nonchlorinated waste contribution(72). It is dangerous to isolate compounds or compound classes and assign toxicity blame since synergistic effects most likely occur between compounds found in mill effluents.

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