

Summary of Case Studies Investigating the Causes of Pulp and Paper Mill Effluent Regulatory Toxicity

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In Canada, effluents from pulp and paper mills are regulated for toxicity. The regulation requires $\geq 50\%$ survival of rainbow trout (*Oncorhynchus mykiss*) exposed to full-strength (i.e., 100%) effluent for 96 h in tests that must be conducted monthly. The regulation also calls for $\geq 50\%$ survival of *Daphnia magna* exposed to 100% effluent for 48 h in weekly monitoring tests. Every year, about 10 to 25% of the mills exceed the regulatory limit at least once in tests with either rainbow trout or *Daphnia magna*. Between 1996 and 2003, we investigated 84 such cases from 32 mills. Of the 84 investigations, 49 involved only trout, 29 involved only *Daphnia*, and six involved both species. We identified the actual cause of toxicity in 70% of the cases and partially or tentatively identified the toxicant(s) in 17% more. In the cases involving only trout, the most frequent causes of toxicity were related to biotreatment performance (e.g., ammonia). In the cases involving only *Daphnia*, the most frequent cause of toxicity seemed to be related to polymeric formulations. For those cases involving both trout and *Daphnia*, the cause of toxicity was traced to more than one compound or to excess concentrations of a specific compound resulting from a spill or biotreatment-related problems.

Key words: toxicity, effluents, paper mills, pulp mills, fishes, invertebrates, regulations

Introduction

In Canada, effluents from pulp and paper mills are regulated for toxicity (Fisheries Act 1992). The regulation requires $\geq 50\%$ survival of rainbow trout (*Oncorhynchus mykiss*) exposed to full-strength (i.e., 100%) effluent for 96 h in tests that must be conducted monthly. The regulation also calls for $\geq 50\%$ survival of *Daphnia magna* exposed to 100% effluent for 48 h in weekly monitoring tests. The trout and *Daphnia magna* tests must be done according to regulatory protocols (Environment Canada 2000a,b).

The Pulp and Paper Research Institute of Canada (Paprican) has tracked the industry's compliance with the toxicity regulation since 1995 (Kovacs and O'Connor 1997; O'Connor and Voss 1998; Kovacs et al. 2002, 2004). Between 1995 and 2000, there has been an improvement in the level of compliance and effluents from most mills have met the regulatory limit. Nonetheless, every year, about 10 to 25% of the mills experienced at least one failure in tests with either rainbow trout or *Daphnia magna*. In 84 such cases between 1996 and 2003, Paprican was asked by mill staff to help identify the cause of toxicity so that appropriate remedial action could be taken for return to compliance as

quickly as possible. This report provides a summary of these investigations.

Methods

Mills Investigated

Effluents from 32 mills were investigated. The mills are categorized in Table 1 according to pulping process and type of biotreatment.

Strategy for Effluent Toxicity Investigations

Overall strategy. Paprican investigations followed episodes or near episodes of non-compliance as reported by the mills' contract laboratories. Instead of using the full toxicity identification evaluation (TIE) protocols developed by the U.S. EPA (Norberg-King et al. 1991; Durhan et al. 1993; Mount and Norberg-King 1993), we used only selected portions, based on the formulation of a hypothesis as to possible cause. In virtually all cases, the investigations were started by discussions with mill staff, which occasionally included site visits to ascertain if recent operating conditions may have influenced the effluent quality. Also, when possible, toxicity test reports and results of relevant chemical analysis of effluents

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TABLE 1. The number of mills, categorized according to process and type of biotreatment systems, for which effluent toxicity episodes were investigated

Pulping process	Type of biotreatment system			
	Activated sludge	Oxygen activated sludge	Aerated lagoon	Sequential batch reactor
Chemical (kraft or sulphite)	2	1	4	0
Mechanical (TMP, CTMP, BCTMP) ^a	10	2	2	0
Multiprocess (chemical + mechanical)	4	3	2	2

^aTMP: thermomechanical pulp; CTMP: chemi-thermomechanical pulp; BCTMP: bleached CTMP.

were reviewed. Usually this was sufficient to formulate a hypothesis about the cause of toxicity. The hypothesis could then be verified on actual effluent samples by selective diagnostic tests from the U.S. EPA protocols (e.g., for ammonia, reduced effluent toxicity by cation treatment) and/or supplemented with chemical analysis for the suspected toxicants (e.g., carbon dioxide, resin acids, metals) according to published methods (APHA, AWWA, WPCF 1998; Voss and Rapsomatiotis 1985). The effluent samples sent to Paprican for diagnostic work were taken fresh or were archived and corresponded to the sample causing toxicity at the mill's contract laboratory. In all cases, we closely monitored symptoms (e.g., trout gasping at the surface) exhibited by the organisms during toxicity tests. In many cases, these were very helpful in leading to the identification of a particular causative agent.

Polymeric formulations. Pulp and paper mills use a variety of additive formulations for flocculation, retention, drainage, strength, sizing and coating. The precise composition of these formulations is typically proprietary, but, they may include polymers (e.g., cationic, anionic or amphoteric polyacrylamide, polyethyleneamine, copolymer of acrylamide and dimethylaminoethylacrylate), latex, wax, defoamers, surfactants and petroleum distillates.

There are no specific toxicity identification tests or routine analytical procedures for polymeric formulations. Therefore we used circumstantial evidence for diagnosis purposes. In the case of effluents affecting *Daphnia*, we used criteria for identification based on our observations from an incident of effluent toxicity (effluent did not cause any trout mortality) caused by an accidental release of a polymeric formulation (paper machine retention aid: cationic copolymer of acrylamide and dimethylaminoethylacrylate in water-oil emulsion) at a mill. The symptoms or criteria included debris attachment to the *Daphnia* carapace (see Fig. 1), evidence of distended or split carapaces, concentration-independent mortality and the elimination of toxicity by filtration (1.2 µm).

Special cases. When the hypothesis-based approach did not lead to any clues concerning the cause of toxicity,

the effluent samples were fractionated with methyl t-butyl ether (MtBE) into solvent and water phases. The solvent phase (containing mostly the non-polar compounds) was evaporated to dryness and the residue was dissolved in water of the same volume as the solvent-extracted effluent. The solvent and water fractions were then tested for toxicity and, depending on which of the two fractions were toxic, were analyzed by gas chromatography (GC) or GC/mass spectrometry (MS).

Toxicity Testing

For diagnostic work, more than half of the toxicity tests with rainbow trout were done with three fish in two-litre volumes as opposed to the regulatory requirement of 10 fish in volumes sufficient to result in a loading density of ≤0.5 g/L (Environment Canada 2000a). In most cases, the regulatory loading density of ≤0.5 g/L was also met in the two-litre tests with three fish. We found that the results of these small-volume trout tests were comparable to the results of regulatory trout tests in over 90% of the cases. The two-litre tests made the manipulations (e.g., filtration, solvent extraction) of effluent samples more manageable. In our experience, the use of smaller surrogate species, such as larval fathead minnows (*Pimephales promelas*), did not provide comparable results to the regulatory trout test to the same degree as the small-volume test. Hence, trout were always used for investigations of effluent toxicity episodes involving rainbow trout.

Toxicity tests with *Daphnia* were done according to regulatory protocols (Environment Canada 2000b), although most tests were not done in replicates. Due to the small volumes required for *Daphnia* testing, there was no need to modify the test protocol or to consider the use of surrogate species.

Results and Discussion

We investigated 84 cases of toxicity episodes involving 32 mills. Clearly, some of the mills had more than one toxicity episode. Of the 84 investigations, 49 involved only trout, 29 involved only *Daphnia* and six involved

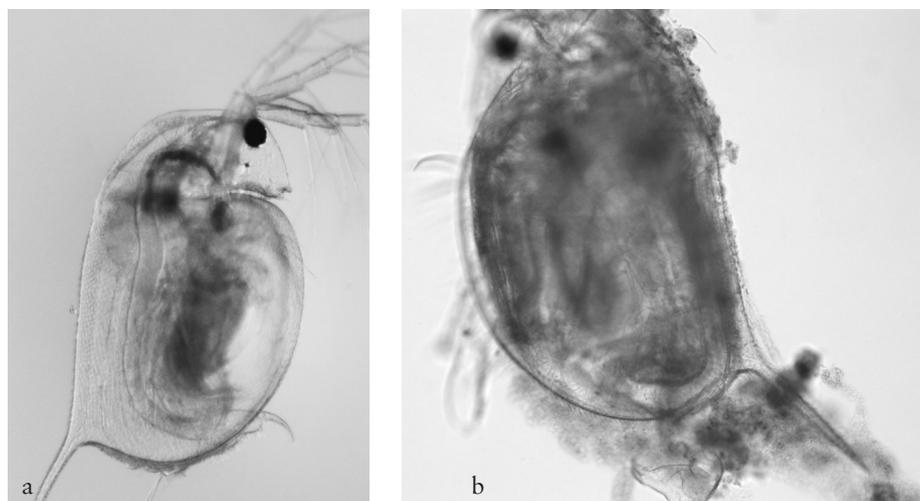


Fig. 1. Pictures of a) normal *Daphnia* and b) *Daphnia* with debris attachment to the carapace (outer shell) as a result of exposure to an effluent suspected to contain a polymeric formulation.

both species, indicating that the causes of toxicity tended to be species specific. Because of this, we summarized the outcome of these investigations separately for each of the two species and for the situations in which both species were affected.

The outcomes of the investigations were classified into one of four categories: i) the effluent did not cause any mortality at Paprican, ii) the cause of toxicity was identified, iii) the cause of toxicity was partially or tentatively identified, and iv) the cause of toxicity was unidentified.

Investigations Involving Effluent Toxicity Only to Rainbow Trout

The summary of the investigations involving effluent toxicity to trout is shown in Fig. 2. In about 20% of the cases (11 of 49 investigations), the effluent sent to Paprican did not cause any mortality. For the 38 investigations where effluent toxicity was evident, the cause of toxicity could be identified ~60% of the time (22 of 38 cases), partially or tentatively identified ~20% of the time (9 of 38) and could not be identified ~20% of the time (7 of 38).

Effluent sample not toxic. The effluent samples were usually sent to Paprican for investigation shortly after the mill's contract laboratory reported toxicity. On eight occasions, when the effluent was re-sampled, there was no mortality noted at Paprican. In these instances, we assumed that the original toxicity episode observed at the mill's contract laboratory was most likely caused by a one-time event, such as a spill or a brief upset of the biotreatment plant. Another possibility was that mortality had occurred because of some laboratory-related problem. On three occasions, an archived sample corre-

sponding to the effluent reported to be toxic by the mill's contract laboratory did not cause any mortality at Paprican. When this happened, in addition to a potential problem at the contract laboratory, there was also the possibility that the cause of toxicity was not persistent as the effluent was tested at Paprican >7 days after sampling.

Cause of toxicity identified. Most (18 of 22) of the identified causes of toxicity to trout were related to biotreatment operating conditions, upsets or general performance. In these cases, the toxicants included ammonia, carbon dioxide, resin acids, or their combination. Based on tests in our laboratory, these compounds are one and a half to four times more toxic to trout than to *Daphnia*. On fewer occasions (4 of 22), toxicity was caused by non-biotreatment related factors such as metals and polymeric formulations.

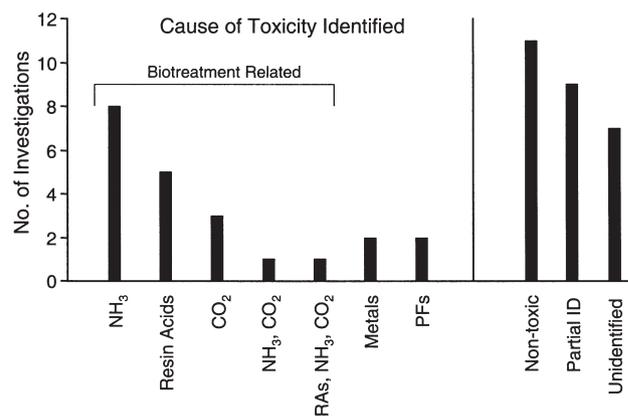


Fig. 2. Summary of investigations of effluent regulatory toxicity episodes involving rainbow trout (RAs: resin acids; PFs: polymeric formulations).

Ammonia. For the biological treatment of the organic material in effluents, most mills need to add nitrogen and phosphorus (McCubbin 1983). The nitrogen addition can result in some residual ammonia in the final discharge. The concentration of total ammonia (un-ionized ammonia and ammonium ion) in the effluent at the point of discharge is usually not sufficient to cause mortality. However, during toxicity testing, due to aeration, the carbon dioxide from the effluent is driven off and this results in an increased pH (Mount and Mount 1992). The exact rate and amount of increase are variable and depend on the carbonate buffering capacity of the effluent. The increasing pH increases the proportion of the un-ionized molecules (pKa 9.6) and the toxicity of un-ionized ammonia (96 h LC50 at Paprican is about 0.4 to 0.6 mg/L) is greater than the ammonium ion (Thurston et al. 1981). As such, trout mortality can occur not only because of the concentration of total ammonia in the effluent but also because of the test condition resulting in increased pH. Trout are about twice as sensitive to ammonia than *Daphnia*.

Correct dosing of nutrient nitrogen to effluent treatment systems is difficult. For high-rate systems, variations in BOD and hydraulic load can lead to variable nitrogen residuals. The operator has to tread a fine line between nutrient deficiency, which leads to poor microbial growth, and excessive residuals, which, in the case of ammonia, can result in toxicity. In aerated lagoon designs or aerated stabilization basins (ASBs), nutrient demand tends to be seasonal due to benthic feedback in the warmer months (Amberg and Bachman 1981). When this happens close to discharge locations, toxicity, due to ammonia, can also occur.

Carbon dioxide. Carbon dioxide-related toxicity is specific to oxygen activated sludge treatment plants. These plants are oxygenated rather than aerated and are sealed to minimize the loss of oxygen (Paice et al. 2003). However, the sealed reactors also limit the escape of carbon dioxide, resulting from the microbial degradation of the organic waste, into the atmosphere. Much of the carbon dioxide will be in solution and, on occasion, the concentration (>125 mg/L) is sufficient to cause trout mortality.

Trout are about one and a half times more sensitive to carbon dioxide than *Daphnia*. Despite this, effluent carbon dioxide may end up causing only *Daphnia* mortality. In trout tests, the effluents are pre-aerated and aerated during the test itself. This results in more rapid loss of carbon dioxide from solution than occurs in *Daphnia* tests which are not pre-aerated and not aerated during the test itself.

Resin acids. Normally, biotreatment plants decrease the levels of resin acids by $\geq 90\%$ (McLeay 1987) so that, in our experience, the concentration in the final discharge is usually ≤ 0.2 mg/L. When the efficiency of the biotreatment system is adversely affected by increased BOD load, changes in pH (Werker and Hall 1999), insufficient

nutrients, or accidental spills, the resin acid concentration may not be reduced sufficiently and this can result in a toxicity episode. This usually happens when the resin acids exceed 1 mg/L. The actual concentration causing mortality will vary depending on pH. Contrary to ammonia, the toxicity of resin acids is decreased with increasing pH (McLeay et al. 1979). *Daphnia* are about four times less sensitive to resin acids than trout.

Metals. For two effluents, metal toxicity, specifically copper and manganese, was mainly due to very low hardness, that is, <40 mg/L as CaCO₃. Metal toxicity increases with decreasing hardness (Pagenkopf 1983). For a TMP mill effluent with a hardness ≤ 20 mg/L as CaCO₃, copper concentrations in the range of 10 to 20 $\mu\text{g/L}$ were found to cause mortality. This effluent was not toxic to *Daphnia magna*, even though copper has been reported to be quite toxic to *Daphnia* as well, in fact sometimes more toxic than to trout (Lankford 1990). However, in *Daphnia* tests, the regulatory protocol (Environment Canada 2000b) requires that hardness be adjusted to 25 to 30 mg/L as CaCO₃. This increase in hardness, as well as the general effluent matrix, may have reduced the potency of copper to *Daphnia*.

In another TMP effluent, with hardness of 20 to 40 mg/L as CaCO₃, trout mortality was traced to a manganese concentration of approximately 2.5 mg/L. This was confirmed by spiking tests in which we added different concentrations of MnCl₂ (Mn²⁺) to a non-toxic TMP effluent with a hardness of 40 mg/L as CaCO₃ and found the 96-h LC50 to be 2.7 mg/L as Mn²⁺ for trout and 18 mg/L for *Daphnia*. This was consistent with the finding that the mill effluent caused mortality only to trout.

Polymeric formulations. The toxicity of polymeric formulations to fish can be attributed mostly to interactions at the gill surface resulting in interference with oxygen and ionic transfer capacity (Murgatroyd et al. 1996). This can result in effects on respiration and osmoregulation. Most polymeric formulations are more toxic to *Daphnia* than to fish, but exceptions can occur. Biesinger et al. (1976) found four of five polyelectrolytes more toxic to *Daphnia* than to rainbow trout. Biesinger and Stokes (1986) reported that eight of 15 cationic polyelectrolytes were more toxic to *Daphnia* than to fathead minnows (*Pimephales promelas*). Of the remaining seven, three were about equally toxic to both species and four were more toxic to minnows. Of 37 polymeric formulations used for wastewater treatment, Hall and Miranda (1991) found 81% were more toxic to *Daphnia pulex* than to fathead minnows. The reversal in trend involved highly charged mannich polymers.

For two effluents we studied, toxicity to trout was traced to polymeric formulations used by the mills as a consequence of atypical release into the wastewater system. In one case, the role of the formulation was verified by effluent spiking tests. The concentration of the formulation tested and found to cause mortality was based

on an estimate of concentration in the effluent following an accidental release. In the other case, the toxicity episode occurred immediately after the sewerage of left-over polymeric formulations. The effluent eventually became non-toxic after the disposal was ceased.

Cause of toxicity partially or tentatively identified. In nine of 38 cases (~20%) where the effluent caused mortality, the cause of toxicity to trout was only partially identified.

Sublation, which involves sparging the effluent with air in a glass cylinder and results in the removal of surface active material from solution (Norberg-King et al. 1991), eliminated the toxicity of two effluents. The material causing toxicity was in the subluted residue remaining in the glass cylinder used for sparging. According to the U.S. EPA TIE manual (Norberg-King et al. 1991), this is a strong indication that the toxicant has surface-active properties. During sparging, surface-active material is brought to the surface with the gas bubbles where they break up and deposit on the sides of the glass cylinder.

For seven effluents, the toxicants were solvent-extractable. One example involved effluent from a TMP mill. The effluent was extracted with MtBE and the toxicity was recovered in the solvent fraction. Gas chromatographic analysis of the MtBE extract showed that a group of peaks (retention time 24–25.5 minutes) were similar to nonyl phenol (see Fig. 3) and this was confirmed by mass spectrometry (MS). The nonyl phenol (NP) concentration was estimated to be ~50 µg/L. The 96-h LC₅₀ of NP to trout has been reported to be between 200 and 300 µg/L (Servos 1999) and, as such,

NP alone could not account for the toxicity. The solvent extract was then washed with base (0.1 N NaOH) to separate its components into a basic water phase and a neutral solvent extract. The toxicity was found to be in the basic water phase and this was analyzed by GC with and without methylation (see Fig. 4). The unmethylated sample indicated that some NP was in the basic fraction although in a very small concentration. In the methylated fraction, several fatty and resin acids were identified. There were also a number of other peaks (retention time 28–29 minutes) and examination of the mass spectral data in this region indicated the presence of what appeared to be a carboxylic acid breakdown product of nonyl phenol ethoxylates (NPEs).

On the basis of this investigation, the toxicity was attributed to organic(s), containing the NP moiety and an acidic functional group, such as carboxylic acid. An additional piece of evidence for this possibility came from symptoms of the fish exposed to the effluent. These included sluggishness, resting on the bottom, and, most distinctive, no response to prodding with a glass rod. These symptoms were also observed in tests with a commercially available nonyl phenol dissolved in water. Overall, while the investigation did not actually identify a specific compound as the cause of effluent toxicity, it did show that biodegradation products of NPEs may be in the effluent (despite the fact that NPE usage at the mill in question was discontinued prior to the toxicity episode) and may have the potential to cause trout mortality. This is consistent with reports that nonyl phenol, a biodegradation product of NPEs, is more toxic than the original NPEs (Servos 1999).

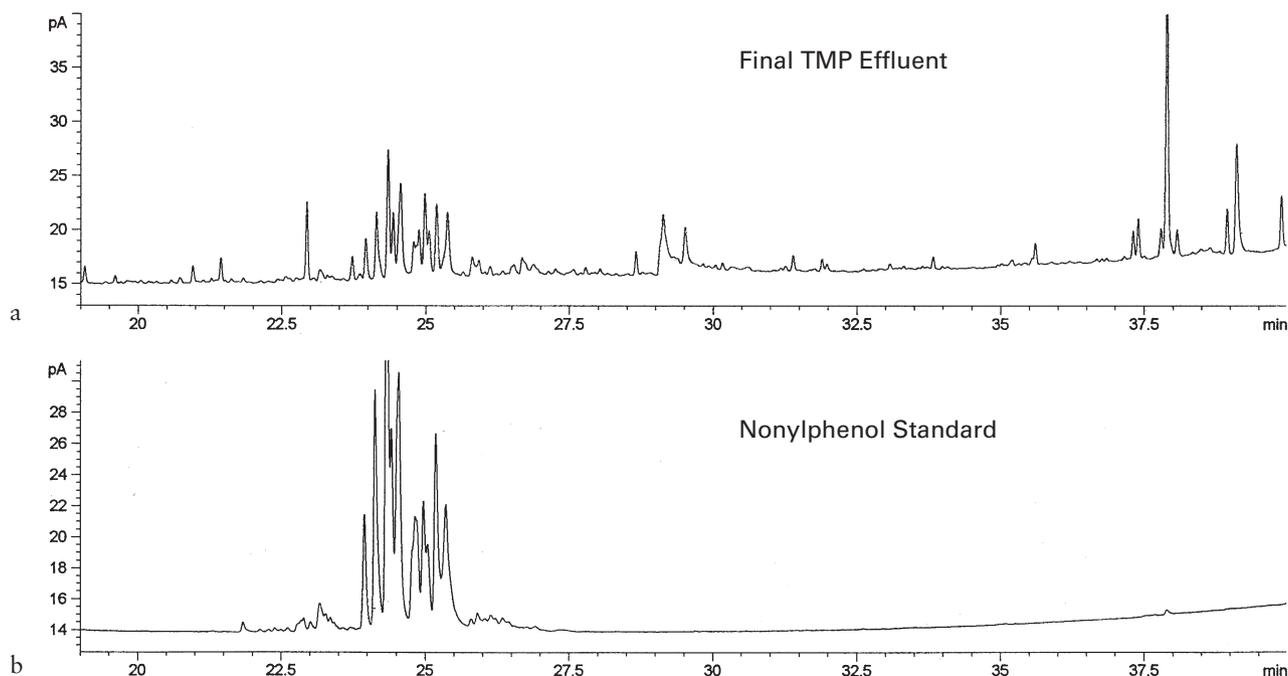


Fig. 3. Gas chromatogram comparison of a) MtBE extract of TMP effluent with b) nonyl phenol standard.

Cause of toxicity unidentified. The cause of toxicity could not be identified for ~20% (7 of 38 cases) of cases. The information from a review of the toxicity test data from the contract laboratories and discussions with mill staff did not lead to suspected candidates. The diagnostic tests provided no clues, although they were helpful in eliminating some common causes of toxicity, such as ammonia and resin acids.

On one occasion, mill staff reported that a TMP effluent exceeding the regulatory limit was found to have an elevated nitrite concentration. Normally, the $\text{NO}_2\text{-N}$ concentration in the effluent from this mill was <0.1 mg/L. During the toxicity episode, the $\text{NO}_2\text{-N}$ concentration reached approximately 5 mg/L, possibly due to overdosing of nitrogen into the biotreatment system. This particular biotreatment system was an aerated lagoon which was periodically problematic due to rapid biomass accumulation in the quiescent zone. Spiking nitrite into a non-toxic TMP effluent indicated that trout mortality can occur at $\text{NO}_2\text{-N}$ concentration near 6 mg/L (*Daphnia* are at least one and a half times less sensitive than trout), but we saw no mortality at 5 mg/L. Nevertheless, it was possible that nitrite at least contributed to effluent toxicity and we felt it worthwhile to at least alert operators of biotreatment systems to this possibility. Nitrite-related toxicity at a TMP/CTMP mill has been reported previously (Servizi and Gordon 1986). Nitrite toxicity is unlikely for bleached kraft mill effluents (BKMEs). The toxicity of nitrite decreases with increasing chloride concentration (Thurston

et al. 1981). The concentration of chloride in TMP effluents can be as low as 10 mg/L, but BKME chloride concentrations are typically between 200 to 400 mg/L. At a chloride concentration of approximately 200 mg/L, the 96-h LC_{50} of nitrite was estimated to be about 100 mg/L on the basis of tests done in our laboratory.

Investigations Involving Effluent Toxicity Only to *Daphnia magna*

The summary of the investigations involving effluent toxicity to *Daphnia magna* is shown in Fig. 5. In about 40% of the cases (13 out of 29), the effluent sent to Paprican did not cause any mortality. For ~90% (14) of the 16 effluents that caused *Daphnia* mortality, the cause of toxicity could be related to polymeric formulations on the basis of criteria described in the methods. The cause of effluent toxicity could not be identified at all or only partially/tentatively identified in two other cases.

Effluent sample not toxic. As for similar situations involving trout, when the effluent sample sent to Paprican did not cause any *Daphnia* mortality, we took this as an indication of a minor mill upset or possible laboratory-related problems. In two of these cases, the mortality observed in the mill contract laboratory could actually be linked to a specific laboratory problem. In one laboratory, there was unacceptably high (>10%) mortality in the population of *Daphnia* held for testing. In the other,

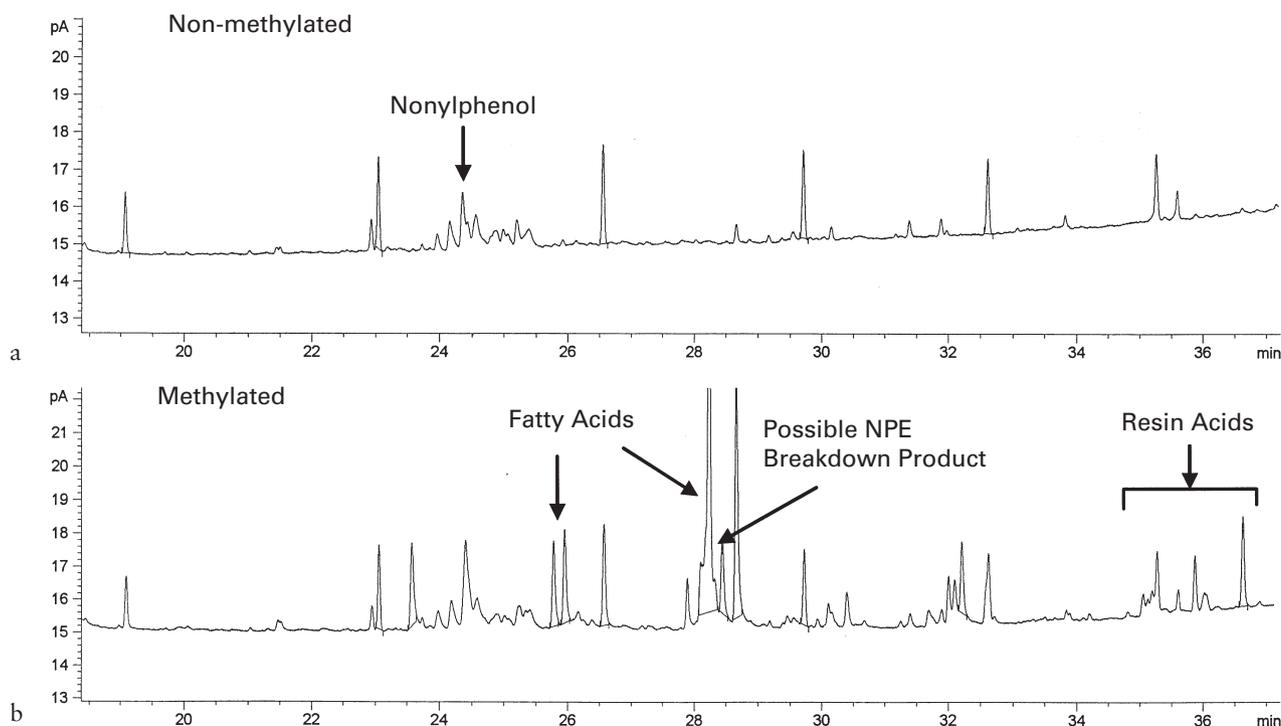


Fig. 4. Gas chromatogram of MtBE extract of basic water phase: a) non-methylated, b) methylated.

the issue was effluent hardness. The regulatory protocol (Environment Canada 2000b) calls for increasing effluent hardness when it is <25 mg/L as CaCO_3 and requires that it be checked if conductivity is <100 $\mu\text{mhos}/\text{sec}$. The conductivity of the sample in question was 1000 $\mu\text{mhos}/\text{sec}$ and this was thought to be indicative of hardness above 25 mg/L as CaCO_3 . While conductivity may be a good indicator of hardness for most effluents, in this case, the high conductivity of the effluent was misleading and the actual effluent hardness of <25 mg/L as CaCO_3 was not adjusted by the contract laboratory.

Cause of toxicity identified. Polymeric formulations were thought to be involved in all cases for which the cause of effluent toxicity to *Daphnia* was identified. This is not surprising given that *Daphnia* are reported to be more sensitive than fish to most of these formulations (Biesinger et al. 1976; Biesinger and Stokes 1986; Hall and Miranda 1991). However, it is important to note that polymeric formulations are not likely to cause effluent toxicity problems when used at the right doses and if accidental spills, when they happen, can be contained. Most effluent toxicity episodes occurred as a result of accidental releases or operational errors in dosing, particularly when there was a switch to the use of a new additive. In the latter case, there was a trial and error period required to optimize dosing.

As with fish, polymeric formulations are thought to exert their effects on *Daphnia* as a result of interaction at their surface. Fort et al. (1992) suggested that polymeric formulations can interfere with the ability of cladocerans (in their study, specifically *Ceriodaphnia dubia*) to regulate ion and water exchange resulting in osmoregulatory distress. This caused *C. dubia* to either dehydrate or bloat. The bloating or distention of the carapace in *Daphnia* is one of the symptoms we used for diagnosing effluent toxicity related to polymeric formulations. With formulations interacting at the surface of *Daphnia*, this could also explain another symptom we

used for diagnosis, that of debris attachment to the carapace (see Fig. 1). Still another unique symptom we used for diagnosis was concentration-independent mortality. Cary et al. (1987) also described such relationships in *Daphnia* tests with cationic polymeric formulations and explained that at higher doses, the ability of the polymeric formulations to interact with the surface of *Daphnia* could be reduced by repulsion of electrostatic charges, hence the occurrence of greater mortality at lower concentrations than at higher concentrations.

Partial/tentative or unidentified cause of toxicity. In one case, based on sublation tests, the toxicant was partially identified as surface-active material. In another case, none of the effluent manipulations tests, such as filtration, or chemical analysis provided any clues as to the cause of toxicity.

Investigations Involving Effluent Toxicity to Both Rainbow Trout and *Daphnia magna*

The summary of the investigations involving both species is shown in Fig. 6. There were only six such investigations and, for two, the effluent samples sent to Paprican did not cause any trout or *Daphnia* mortality. The causes of toxicity for the other four effluents were identified to be stearic acid, resin acids, resin acids in combination with some surface-active material, and a combination of carbon dioxide and total reduced sulfur (TRS) compounds, in particular, dimethyl disulphide (DMDS).

The stearic acid episode resulted from the use of a floor cleaning product in excessive amounts. The material ended up in the wastewater system and caused toxicity to both species. The resin acid and resin acid/surface-active material related toxicity episodes occurred when a mill was experiencing problems with its biotreatment system. In this case, the resin acid concentration reached 9.3 mg/L and therefore was high enough to affect both species.

The carbon dioxide/DMDS related toxicity was the consequence of effluent treatment in a sealed oxygen activated sludge plant. Because such systems are sealed, carbon dioxide, and volatile TRS compounds, such as DMDS originating from the condensates of kraft mills, will remain in solution and may result in mortality (Paice et al. 2003) when critical concentrations (e.g., DMDS concentrations of 10 and 50 mg/L for trout and *Daphnia*, respectively) are exceeded. We believe that there are several aggravating factors that can result in toxicity of effluents from oxygen activated sludge treated effluents. One is the control of the oxygen vent purity which influences the flow of gases out of the treatment system. If the vent purity is very low, then the headspace gas has a longer residence time in the reactor and volatile gases tend to accumulate, resulting in a higher equilibrium concentration in the liquid phase.

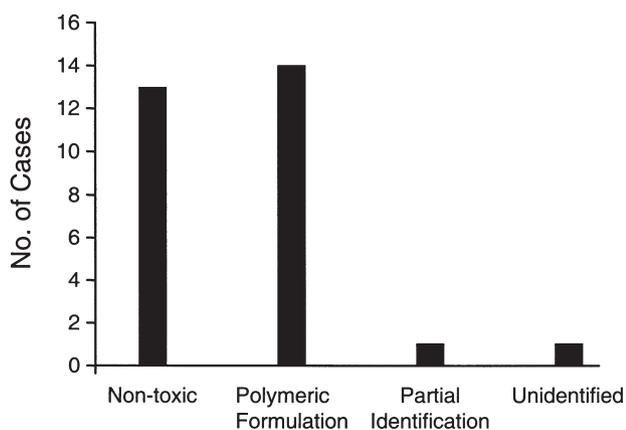


Fig. 5. Summary of investigations of effluent regulatory toxicity episodes involving *Daphnia magna*.

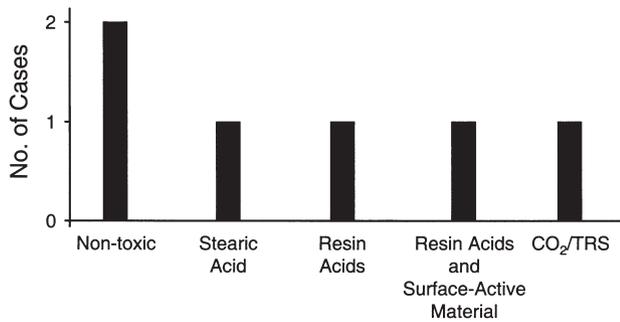


Fig. 6. Summary of investigations of effluent regulatory toxicity episodes involving both rainbow trout and *Daphnia magna* (TRS: total reduced sulfur compounds).

Toxicity Episodes in Relation to Mill Process and Biotreatment

The most frequent causes of effluent toxicity involving trout could be traced to the biotreatment system performance (e.g., overload, spills), whereas, the most frequent causes of toxicity involving *Daphnia* seemed to result from the accidental release or misapplication of polymeric formulations. Could these, in turn, be related to a specific biotreatment system or mill process?

Any episode involving volatile compounds, such as carbon dioxide or DMDS, will only occur at mills with sealed oxygen activated sludge treatment. The possibility of resin acid related toxicity will also be greater for these types of effluents as the pH tends to be between 6 and 6.5, as opposed to ≥ 7 for effluent from most other types of biotreatment systems (Paice et al. 2003). Resin acids are more toxic to aquatic organisms at lower pH (McLeay et al. 1979). For ammonia, of the eight cases we investigated, seven occurred with effluents from activated sludge systems and only one was from an ASB. This may indicate that nutrient dosing is more of a problem for activated sludge treatment or it could just be a reflection of the fact that the activated sludge treatment is about twice as common as ASBs in Canada.

Concerning the toxicity of effluent to *Daphnia* that may have been caused by polymeric formulations, 12 of the 14 cases involved mills making mechanical pulp, one involved a multiprocess mill and one involved a kraft mill. All 14 mills produced paper and all had activated sludge treatment. Papermaking additives (e.g., retention aids) were frequently suspected to be the cause of toxicity involving *Daphnia*. It appears that once such material gets into an activated sludge treatment system it may be very persistent because a portion of the sludge is re-circulated.

Other Liquid Discharges

The toxicity regulation applies to all liquid mill discharges. For some mills, this includes non-process or

cooling water that is discharged separately from the process effluents. Between 1996 and 2003, we investigated 12 toxicity episodes caused by cooling waters. Four of the episodes involved only rainbow trout. Of these, two were attributable to copper (water hardness about 5 to 10 mg/L as CaCO₃), one to contamination from the runoff of a metal roof, and one to total residual chlorine (TRC). Five toxicity episodes involved only *Daphnia magna*. In one case, the cooling water was non-toxic when tested at Paprican. In another, toxicity was both marginal and non-persistent so that the diagnostic tests applied provided no information. In a third, our investigation yielded no known cause. The cause of toxicity for the remaining two cases was identified as TRC. Three of the toxicity episodes involved both trout and *Daphnia*. In one of these cases, the effluent was only toxic to *Daphnia* when tested at Paprican and mortality was attributed to contamination by oil. In the other two cases, TRC was responsible for mortality to both organisms. Residual chlorine, previously identified as the most likely cause of toxicity in cooling waters (Kovacs et al. 2000), continued to be the primary cause of trout and *Daphnia* mortality in such mill discharges. Though metals were also identified as a cause of toxicity in some cooling waters, again this was because of the use of very soft water.

Summary and Conclusions

- Between 1996 and 2003, Paprican investigated 84 cases of effluent regulatory toxicity episodes involving 32 mills. Of the 84 investigations, 49 involved only trout, 29 involved only *Daphnia* and six involved both species.
- For ~30% (26 of 84) of the reported cases of toxicity episodes, the samples tested by Paprican were not found to cause mortality. This indicated that the toxicity episodes reported by the mill's contract laboratories were not persistent or were the outcome of brief mill upsets. It also indicated the possibility of some problem with the test species or test conditions in the contract laboratory.
 - This suggests that investigations for cause of toxicity may not be required or even possible for about one third of the cases.
- The overall success rate in identification of the cause of toxicity for the cases with effluents that caused mortality was about 70% (40 of 58) and for about 17% of the cases (10 of 58) it was possible to make partial or tentative identification of the causative agent(s).
- For the 49 cases involving only trout, the most frequent causes of toxicity were related to biotreatment operating conditions, upsets or general performance. The causative agents were ammonia, carbon dioxide and resin acids. These compounds are more toxic to trout than to *Daph-*

nia. Other causes of toxicity included metals (copper, manganese) when the effluent hardness was <40 mg/L as CaCO₃, polymeric formulations and, possibly, biodegradation products of additives.

- For the 29 cases involving only *Daphnia*, the most frequent cause of toxicity seemed to be related to polymeric formulations. Most episodes occurred as a result of an accidental release or errors in dosing. Polymeric formulations are not likely to cause effluent toxicity problems when used at the right dose and if accidental spills are contained. By and large, these formulations tend to be less toxic to fish than to *Daphnia*, although exceptions can occur.
- For the six cases involving both trout and *Daphnia*, the cause of toxicity was traced to more than one compound (e.g., carbon dioxide and DMDS), to a cleaning product and to a malfunctioning of the biotreatment system resulting in high resin acid concentration in the final discharge.
- Mill process and type of biotreatment system can have an influence on the nature and frequency of the cause of effluent toxicity. For biotreatment systems, activated sludge designs are twice as common as ASBs. Thus, statistically there is a higher potential for reported problems from activated sludge.
 - Toxicity caused by easily spargable compounds, such as carbon dioxide, will only occur at mills with oxygen activated sludge treatment.
 - Ammonia-related toxicity was most frequent for mills with activated sludge treatment.
 - *Daphnia* mortality caused by polymeric formulations occurred only at mills that make paper, mostly at mechanical pulp mills, with activated sludge treatment.

Acknowledgements

We thank Ken Doe (Environment Canada, Moncton, N.B.), Lesley Novak (Stantec, Guelph, Ont.) and Sandra Whiteway (Jacques Whitford Environmental Laboratories, St. John's, Nfld.) for help with the identification of manganese as the source of toxicity for a TMP effluent. We also appreciate the manuscript review and comments by Joseph Sullivan of Paprican.

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- Received: November 25, 2003; accepted: March 22, 2004.