Does an Association between Pesticide Use and Subsequent Declines in Catch of Atlantic Salmon (Salmo salar) Represent a Case of Endocrine Disruption?

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Historical aerial applications of the insecticide Matacil 1.8D provide an opportunity to look for potential effects of the endocrine disrupting compound 4-nonylphenol (4-NP) on Atlantic salmon (Salmo salar) populations. Matacil 1.8D contained the carbamate insecticide aminocarb, with 4-NP as primary solvent. Between 1975 and 1985 Matacil 1.8D was applied to forests in Atlantic Canada to control damage from the spruce budworm (Choristoneura fumiferana). After spraying, estimated concentrations of 4-NP in water fell within a range in which estrogenic effects might be anticipated. The spraying coincided with final stages of smolt development in salmon. Salmon catch data were evaluated considering effects on survival of the smolt stage. There was a significant negative relationship between the returns of salmon and the proportion of tributaries sprayed within the Restigouche River drainage basin in 1977. There was also a broader event of unusually heavy salmon smolt mortality in 1977, which contains a significant relationship indicating that where Matacil 1.8D spraying occurred, the smolt mortality increased. For 16 rivers exposed to spraying between 1973 and 1990, a significant proportion (p<0.005) of the lowest salmon catches coincided with Matacil 1.8D spraying. A decline coinciding with the use of Matacil 1.8D was also apparent in blueback herring (Alosa aestivalis) catches in New Brunswick. Because similar relationships were not evident for Matacil 1.8F or fenitrothion, neither of which were formulated with 4-NP, we hypothesize that the 4-NP in Matacil 1.8D was the causal agent. Concentrations of 4-NP described here are within current ranges encountered in industrial effluents and municipal sewage outfalls. Key words: aminocarb, endocrine disruptor compounds, 4-nonylphenol, populations, salmon, smoltification. Environ Health Perspect 107:349–358 (1999). [Online 24 March 1999] http://ehpnet1.niehs.nih.gov/docs/1999/107/p349-358fairchild/abstract.html

In northeastern North America, a large-scale program of aerial forest spraying with insecticides to reduce damage from the spruce budworm [Choristoneura fumiferana (Clemens)] has been under way for decades. DDT was applied in the 1950s and 1960s, and fenitrothion and aminocarb were applied in the 1970s and 1980s. Use of Bacillus thuringiensis (Bt) was more common in later years and into the 1990s (I). The use pattern we highlight is that of aminocarb in its formulation as Matacil 1.8D. This formulation contained 4-nonylphenol (4-NP), a known xenoestrogen (2,3), as a primary solvent at about three times the weight (182 g/ha) of the active insecticidal ingredient aminocarb (usually 70 g/ha) (4). Matacil 1.8D was applied to New Brunswick in test areas in 1971 and 1972, and was then used in the full-scale spray program from 1975 to 1985 across Quebec, New Brunswick, and Newfoundland (1,4). After 1980, Matacil was changed to a water-based flowable formulation in New Brunswick, when concerns were expressed that measured stream concentrations of Matacil 1.8D were too near the threshold for acute toxicity to fish due to the presence of 4-NP (5–7). The provinces of Quebec and Newfoundland continued to primarily use the Matacil 1.8D formulation into the mid-1980s. Extensive areas were sprayed in many years, with a peak combined program (all jurisdictions) in excess of 2 million hectares (1,4).

Because there are well-studied populations of Atlantic salmon (Salmo salar Linnaeus) in the areas where Matacil 1.8D was applied, it is not unreasonable to expect that if detrimental effects of Matacil 1.8D exposure occurred, they might be detected as changes in the salmon catch data. Atlantic salmon is an anadromous species with a complex life history. Eggs are deposited in freshwater in nests in the gravel bottom of rivers during late October and early November. Upon hatching, Atlantic salmon go through four stages in freshwater: alevin, fry, parr, and smolt. This process usually requires 2–3 years. After reaching a critical size, the stream-dwelling parr undergo metabolic transformation into smolts (8,9), the process during which they become physiologically adapted for life in sea water. Around the smoltification period, the developing salmon also imprint to their home stream (10). Smolts hold in pools or in the mouth of the river before migrating to sea during May and June. Once the smolts have left the freshwater river system, population assessment at sea is difficult. After 1 or more years at sea, Atlantic salmon typically return to their native river to spawn. Salmon that return after one winter at sea are referred to as 1SW (one sea winter) fish and would be counted as small salmon or grilse in the recreational catch. Those that return for the first time after 2, 3, or more years at sea (2SW and 3SW fish) are called large salmon. Some repeat spawning occurs; however, the percentage is typically <10% (11). A single or repeated pulse dose of Matacil 1.8D could be expected in many salmon streams between mid-May and mid-June, depending on the year and timing of the spray program to match the local development of the spruce budworm larvae. This timing places Matacil 1.8D in the streams and rivers coincident with the latter stages of parr–smolt transformation. In this paper we describe evidence of a relationship between the use of Matacil 1.8D in Atlantic Canada and historical declines in catches of Atlantic salmon. The association is evaluated on both a local and regional basis with respect to increased mortality of salmon smolts subsequent to forest spraying. The evidence is summarized according to epidemiological criteria, and information from commercial blueback herring (Alosa aestivalis (Mitchill) (gaspereau)) catches in New Brunswick is potentially corroborative. Our findings imply a possible historical case of endocrine disruption that depressed subsequent fish catch. This information also has possible consequences for anadromous fish exposed to estrogens and their metabolites.
Materials and Methods

Forest spray maps were obtained from maps of the Canadian Wildlife Service in Sackville, New Brunswick. Annual Reports of the Forest Pest Control Forum, and various provincial monitoring reports (Table 1) (4,12–30). Information on Atlantic salmon was obtained from Canadian Atlantic Fisheries Scientific Advisory Committee research documents, Department of Fisheries and Oceans Canada (DFO) technical and manuscript reports, DFO Atlantic Fisheries Research Documents, DFO Stock Status reports and research journals (Tables 2–4) (31–51). These data sources are used to manage salmon stocks in eastern Canada.

The overlap for information about forest spraying and availability of information on salmon stocks was best for New Brunswick and Newfoundland, although significant spraying occurred in Quebec. When spraying in Quebec influenced drainage basins shared with New Brunswick, we included the Quebec program. There are probably other places where the effects on salmon could be assessed, such as Anticosti Island in the Gulf of St. Lawrence; however, we could not locate sufficient information to carry out a reasonable analysis. Thus, our emphasis is on the salmon populations in the rivers of New Brunswick and Newfoundland. Our approach was to find the best defined occasions where information allowed for a clear analysis and discuss them in detail. We will present cases from specific locations and times and an integration of wider events between 1975 and 1985. Various types of spray programs took place in the Atlantic region. Forests were sprayed according to the intensity of spruce budworm infestation (Fig. 1) (52). Insecticides were sprayed over large areas in blocks ranging in size from <400 ha to >150,000 ha. Exposure of salmon rivers was determined by overlaying a map of rivers and watersheds on spray maps.

Restigouche River 1977. For the Restigouche River system in 1977, the percent of a drainage basin sprayed with Matalic 1.8D and the percent survival of large salmon was analyzed using linear regression analysis. The percent drainage basin sprayed was determined using digitized New Brunswick and Quebec forest spray maps with areas measured by an AutoCAD program (Autodesk, Inc., Sausalito, CA). The percent sprayed is a ratio of spray block area falling within the drainage basin of the river and the total area of the drainage basin. The percent survival of large salmon (2SW) was determined by comparing the recreational catch of large salmon in the year of question with the average for the previous 5-year period. This method of expressing reductions is similar to that used by Ritter (31) and is commonly used for stock assessment comparisons.

The Main Restigouche River has five major tributaries: Matapedia, Patapedia, Kedgwick, Little Main Restigouche, and Upsalquitch. Recreational catch data are available for each tributary and for the Main Restigouche (43,53). We could not include recreational catch data from the Main Restigouche in the analysis because the numbers reflect the returns to the entire drainage basin. Salmon must move through the Main Restigouche to reach the tributaries. The Main Restigouche produces <10% of the fish in the drainage basin and yet has the largest catch records (43). This does not allow us to relate the numbers caught in the Main Restigouche with a specific exposure. Data from the Little Main Restigouche could not be included because reported catches from there had been combined with the Main Restigouche catches prior to 1982. Salmon runs in the Restigouche are mainly 2SW fish, although some rivers and years would also have low contributions from 3SW fish (54).

Regional salmon catch in 1977. To test the association between recreational catch of small (1SW) and large (2SW, 3SW) salmon in Atlantic salmon rivers and the extent of Matalic 1.8D exposure in 1977 (31), we used a Spearman rank correlation (55). Exposure was determined from spray maps by defining the drainage basin of the river or salmon assessment area and identifying any spray blocks, regardless of size, contained

| Table 1. Summary of operational forest spraying with Matalic in the Atlantic Region (ha) |
|-----------------------------------------------|
| Year | New Brunswick | Newfoundland | Quebec | Maine | Total amount of Matalic 1.8D sprayed |
|------|----------------|---------------|--------|-------|----------------------------------|
| 1972 | -              | -             | D 31,100 (4) | -     | 31,100                            |
| 1973 | -              | -             | D 132,000 (4) | -     | 132,000                           |
| 1974 | -              | -             | D 485,000 (4) | -     | 485,000                           |
| 1975 | D 64,000 (4)  | -             | D 524,600 (4) | -     | 589,600                           |
| 1976 | D 315,000 (4) | -             | D 1,707,912 (12) | D 60 (4) | 2,022,972                         |
| 1977 | D 517,000 (4) | D 58,140 (4,13) | D 1,265,951 (14) | D 120 (4) | 1,841,201                         |
| 1978 | D 755,520 (19) | D 376,604 (4,13) | D 1,010,000 (4) | D 1,200 (4) | 2,143,324                         |
| 1979 | D 1,552,990 (15) | -             | D 565,935 (16) | -     | 2,118,925                         |
| 1980 | D 243,750 (15) | -             | D 55,242 (17) | -     | 236,992                           |
| 1981 | D 238,063 (13) | D 679,694 (18) | -     | -     | 917,757                           |
| 1982 | F 50,790 (15)  | D 43,109 (13) | D 1,256,305 + | F 10,313 (13,20) | 1,299,414                         |
| 1983 | F 121,670 (15) | D 66,554 + F 6,260 (13) | D + F 1,027,356 (27) | -     | 580,232                          |
| 1984 | F 622,500 (15) | D 23,226 (13) | D 325,159 + F 64,351 (22) | -     | 348,385                          |
| 1985 | F 471,250 (15) | -             | -     | -     | -                                 |
| 1986 | F 212,550 (15) | -             | -     | -     | -                                 |
| 1987 | F 17,490 (15)  | -             | -     | -     | -                                 |

Abbreviations: D, Matalic 1.8D (containing nonylphenol); F, Matalic 1.8F.

*Estimate made assuming that half of the Quebec program was the D formulation.

| Table 2. Spearman rank correlation test of Matalic 1.8D exposure and Atlantic salmon catch reduction |
|-----------------------------------------------|
| Location | Exposurea | Rank of exposure | Change in salmon populationb | Rank of reduction |
|-----------|------------|------------------|-----------------------------|------------------|
| Restigouche (small)c | K-Restigouche-others | 11.5 | -45 | 6 |
| Restigouche (large)d | K-Restigouche-others | 11.5 | -77 | 3 |
| Gaspé (small) | Restigouche-Gaspé-others | 9.5 | -51 | 4 |
| Gaspé (large) | Restigouche-Gaspé-others | 9.5 | -78 | 2 |
| Area M | 18.75 | 5 | -43 | 7 |
| Area L | 55.67 | 8 | -17 | 9 |
| Area K (small) | 427.06 | 13.5 | -31 | 8 |
| Area K (large) | 427.06 | 13.5 | -86 | 1 |
| Area J1 | 15.02 | 4 | 13 | 14 |
| Area I | 6.67 | 1 | 3 | 11 |
| Area D | 10.00 | 3 | -2 | 10 |
| Area C | 41.12 | 7 | 8 | 12.5 |
| Area B | 22.2 | 6 | 8 | 12.5 |
| Area A | 9.22 | 2 | -48 | 5 |

r = -0.5626*

*Exposure was determined by the weighted paper method for Areas A–M. Exposure for the Restigouche and Gaspé was determined digitally through use of an AutoCAD (Autodesk, Inc., Sausalito, CA), and then ranked relative to the exposure of Area K.

**Data from Ritter (31).**

*Small salmon.

**Large salmon.

*p<0.025.
within its boundaries. All drainage basins were cut out of paper copies of maps and weighed to determine their mass. For determining spray exposure in the Restigouche, Gaspé, and Area K, the spray blocks were also cut out of paper copies of the spray maps and weighed to determine their mass. The mass of the spray blocks was then multiplied by the number of times spray applications were applied. Exposure was then expressed as the ratio of the mass of the spray blocks (corrected for number of times sprayed) to the mass of the drainage basin. Because details of spray blocks for salmon assessment areas A–M (Newfoundland) were not shown on the maps, this approach was not possible. Alternatively, exposure was represented by the number of hectares sprayed, as indicated by the spray maps, multiplied by the number of applications applied. For this calculation, spray blocks in forest improvement areas were estimated to average 400 ha and sprayed twice. Exposure was then expressed as the ratio of the application-corrected number of hectares sprayed to the mass of the drainage basin.

Because exposure to Area K was determined using both methods, exposure of the Restigouche and Gaspé areas could be estimated relative to the salmon assessment areas in Newfoundland. Reduction data for Atlantic salmon includes all recreational catches of large and small salmon where available from Ritter (31). Exposure is ranked from smallest to largest (1–12), whereas change in population is ranked from largest to smallest (1–12) reduction.

Spray events between 1973 and 1990. Other spray events between 1973 and 1990, for which we had sufficient information to evaluate the effects of exposure to Matacil 1.8D, are summarized in Table 3. Events to be included in Table 3 were screened against five criteria:

- The behavior of the salmon in a given river had to be coherent with exposure. For example, smolts in the upper reaches of large rivers would have to move toward the sea early to reach the river mouth in late spring. They would then miss exposure to a spray event in the

![Figure 1. Extent of eastern North America spruce budworm infestation at about its peak in 1975, and the distribution of salmon rivers in the same area.](image)

### Table 3. Reduction in total returns or recreational catch over 11 years for 16 rivers exposed to Matacil 1.8D spray

| River          | Salmon class | Exposure year | Year of expected effect | Percent of 5-year mean catch | Greatest reduction | Second greatest reduction | Third greatest reduction | Fourth greatest reduction | Fifth greatest reduction | Rank of catch reduction | Years used in ranking |
|----------------|--------------|---------------|-------------------------|-----------------------------|-------------------|--------------------------|--------------------------|--------------------------|-------------------------|------------------------|------------------------|
| Newfoundland   |              |               |                         |                             |                   |                          |                          |                          |                         |                        |                        |
| Harry’s (32)   | 2SW          | 1977          | 1979                    | 3.8                         | 1979              | 1975                     | 1984                     | 1974                     | 1976                    | 1                      | 1974–1984              |
| Beaver (33–39) | 1SW          | 1978          | 1979                    | 198.2                       | 1984              | 1983                     | 1975                     | 1976                     | 1982                    | 8                      | 1974–1994              |
| Campbellton (33–40) | 1SW      | 1978          | 1979                    | 3.9                         | 1978              | 1982                     | 1983                     | 1984                     | 1976                    | 1                      | 1974–1984              |
| Conne (41)     | 1SW          | 1978          | 1979                    | 54.8                        | 1979              | 1977                     | 1983                     | 1978                     | 1984                    | 1                      | 1974–1984              |
| Gambo (33–40)  | 1SW          | 1978          | 1979                    | 51.7                        | 1979              | 1982                     | 1984                     | 1978                     | 1984                    | 1                      | 1974–1984              |
| New Bay (33–39) | 1SW         | 1978          | 1979                    | 189.7                       | 1975              | 1963                     | 1983                     | 1976                     | 1984                    | 1                      | 1973–1993              |
| Northeast Brook (33–39) | 1SW   | 1978          | 1979                    | 152.6                       | 1975              | 1976                     | 1978                     | 1980                     | 1974                    | 10                     | 1973–1983              |

Abbreviations: 1SW, one sea winter fish; 2SW, two sea winter fish. Ranks were used for a Kolmogorov-Smirnov test.

| Big Salmon (45–48) | 1SW | 1979–1980 | 1.1 | 1980 | 1975 | 1977 | 1983 | 1984 | 1 |

### Table 4. Reduction in recreational catch over 11 years for five rivers exposed to Matacil 1.8F spray

| River          | Salmon class | Exposure year | Year of expected effect | Percent of 5-year mean catch | Greatest reduction | Second greatest reduction | Third greatest reduction | Fourth greatest reduction | Fifth greatest reduction | Rank of catch reduction | Range of years in catch |
|----------------|--------------|---------------|-------------------------|-----------------------------|-------------------|--------------------------|--------------------------|--------------------------|-------------------------|------------------------|------------------------|
| Big Salmon (45–51) | 1SW | 1984–1985 | 87.5 | 1980 | 1987 | 1988 | 1986 | 1983 | 7 | 1978–1988 |
| Little Southwest | 2SW          | 1984–1985 | 304.1 | 1983 | 1982 | 1991 | 1987 | 1989 | 10 | 1981–1991 |
| Miramichi (44)   | 2SW          | 1984–1985 | 199.9 | 1991 | 1990 | 1983 | 1981 | 1989 | 9 | 1981–1991 |
| Northwest Miramichi (44) | 2SW      | 1984–1985 | 1986 | 1991 | 1990 | 1988 | 1987 | 1989 | 9 | 1981–1991 |
| Sevogle (44)     | 2SW          | 1985–1987 | 119.2 | 1991 | 1990 | 1983 | 1982 | 1989 | 7 | 1982–1992 |
| Little Southwest | 2SW          | 1985–1987 | 119.1 | 1991 | 1990 | 1983 | 1992 | 1989 | 7 | 1982–1992 |
| Sevogle (44)     | 2SW          | 1985–1987 | 86.3 | 1991 | 1990 | 1988 | 1987 | 1989 | 4 | 1982–1992 |
| Keedgwick (43)   | 2SW          | 1988–1989 | 235.5 | 1993 | 1990 | 1984 | 1985 | 1995 | 11 | 1983–1993 |

Abbreviations: 1SW, one sea winter fish; 2SW, two sea winter fish. Ranks were used for a Kolmogorov-Smirnov test.
upper reaches of a river (e.g., Tobique River, New Brunswick).

- Rivers were excluded if they had physical barriers to salmon migration, such as jams or waterfalls near the river mouth (e.g., Nepisiquit River, New Brunswick).
- Rivers that had major shifts in the pattern of local commercial fisheries were not included (e.g., certain years on the Miramichi River, New Brunswick).
- Specific salmon statistics (a record of catch or a counting fence) were essential to the analysis; therefore, if they were lacking, the river could not be used. This included rivers where no information was available, as well as those large rivers where separate information was not available for each tributary. The tributaries may have experienced different exposures that could not be evaluated independently. Available salmon catch data had to have a mean annual catch of at least 80 fish. The 80-fish criteria was required as a cutoff to ensure consistent catch records over the 11-year periods evaluated; many of the smaller rivers with lower mean returns normally have high fluctuations in catch, which could mask effects due to specific events.

- A spray event was included in the analysis when greater than 20% of a river drainage basin was sprayed. Based on the information obtained from the previous analysis of the Restigouche tributaries, an area comprising at least 20% of the total basin was required to depress subsequent catch records. When a drainage basin was repeatedly sprayed, only the years when the more significant exposures took place were used in the analysis.

The year of expected effect was predicted for rivers and years for which we found a significant exposure, and adjusted to account for the predominant habits of the biological component of the particular salmon fishery (i.e., whether mainly 1SW, 2SW, or 3SW returns). This approach, consistent with Ritter (31) in his assessment of the 1977 smolt mortality, grouped returning fish as small (mostly 1SW) and large (2SW or 3SW) fish. The year of expected effect was then used to define an 11-year period of comparison for each river, which included the year of expected effect, the 5 years before, and the 5 years after. Change in catch was calculated by expressing the catch of the year of expected effect as a percentage of the mean of the previous 5 years. When 5 years of data were unavailable before the year in question, the closest 5 years were used. Changes in catch were calculated for each spray event in the same manner for all 11 years and ranked according to all reductions calculated over the period. To assess the probability of predicting a reduction based on an exposure as compared to the probability of predicting a reduction by chance, a Kolmogorov-Smirnov goodness-of-fit test was used on the rank data to test for a random distribution of predicted events (56).

**Other spray formulations.** In addition to the Matal 1.8D exposures, we gathered a set of exposure events for both Matal 1.8F (flowable formulation of Matal without 4-NP) and fenitrothion. These were assessed by the same five criteria as described above for the Matal 1.8D spray events. For Matal 1.8F, we obtained a set of eight events from five rivers that had been sprayed in 1984, 1985, or 1986. For fenitrothion, we obtained a set of 16 events from nine rivers in New Brunswick that had been sprayed in 1976, 1977, or 1978.

**Other salmon life stages.** While compiling data on salmon catch, we also gathered information from the same assessment reports on fry and parr and their abundance and assessed the presence or absence of association in data tables from numerous New Brunswick rivers over time.

**Blueback herring.** The biology of the blueback herring [Alosa aestivalis (Mitchill)] has similarities to that of Atlantic salmon. Blueback herring are anadromous, entering freshwater to spawn. In the Miramichi River, the blueback herring contributes to the gaspereau fishery, which includes both blueback herring and alewives [Alosa pseudoharengus (Wilson)]. Blueback herring return to the river from late May to mid-June, and spawn in flowing water, while alewives arrive earlier and tend to move up into lakes and pools to spawn. Larval blueback herring hatch in 3-6 days and move downstream to the sea over the next few weeks (57). Like salmon smolts, young blueback herring will experience an osmoregulatory change as they move from fresh to salt water. They recruit to the fishery typically at 4-5 years of age (58-63). Effects on blueback herring were assessed in the Miramichi River, following a possible exposure to Matal 1.8D in 1979. Fishery statistics were examined, focusing on the commercial catch in 1984, which had a fishing season from 26 May to 15 June. To make the catch data of 1984 comparable with those of other years (1981-1987), all other catches were adjusted to include only data for the same period.

**Results**

**Restigouche River 1977.** Recreational catch numbers from four Restigouche River tributaries (Matapedia, Patapedia, Kedgwick, and Upsalquitch) were included in our analysis because the numbers pertain to each river and we can determine a specific magnitude of spray exposure within the drainage basin of each tributary (14.64) (Fig. 2). When the percentage of each drainage basin sprayed with Matal 1.8D in 1977 was regressed against the returns of 2SW fish to each tributary in 1979, there was strong linear relationship (Fig. 3).

**Regional salmon catch in 1977.** There was high mortality of salmon from the 1977 smolt class (32), which was a pan-population event that affected most salmon rivers in the Atlantic region. To broaden the geographic scale for analysis of the potential effects of Matal 1.8D spraying, we combined Ritter’s estimates of reductions in salmon catches (31) with estimates of exposure, where possible. The recreational catch data for areas discussed by Ritter (31) for which we could assign an estimate of exposure are summarized in Table 2. This subset of data was used for the Spearman rank correlation test. Within the 1977 data for smolt mortality, there was a significant relationship (Spearman rank correlation, r=0.025), which suggested that where Matal 1.8D was sprayed, the smolt mortality increased (Table 2). Reductions in salmon recreational catch were also observed in some other areas where we were unable to assign an exposure.
**Spray events between 1973 and 1990.**

For rivers in Newfoundland and New Brunswick, 9 of the 19 events where Matacil 1.8D exposure was identified coincided with the greatest reductions in catch observed over an 11-year period in each river (Table 3). Sixteen of the 19 events ranked within the first to sixth greatest reductions of a possible rank of 1–11. This distribution of ranks was found to be significantly different from random, using a Kolmogorov-Smirnov goodness-of-fit test (d_{max} = 0.54; p<0.005). Five of the 19 events were from exposure in 1977 and were included in results discussed above for the Restigouche River and the broader smolt mortality. However, even if these five events are removed from the analysis, the Kolmogorov-Smirnov test remains significant (d_{max} = 3.36; p<0.05).

**Other spray formulations.**

When we examined catch data for relationships involving possible effects on smolts and exposure to the flammable aminocarb formulation, Matacil 1.8F (Table 4), or fenitrothion (data not shown), we were unable to find relationships similar to those observed for Matacil 1.8D. The Kolmogorov-Smirnov test for Matacil 1.8F was not significant (d_{max} = 3.63; p<0.50).

**Other salmon life stages.**

When we examined the catch data and considered possible effects of Matacil 1.8D on survival of Atlantic salmon fry or parr, no relationships were apparent (data not shown).

**Blueback herring.**

Recruitment of blueback herring to the commercial gaspereau fishery from 1983 to 1985 generally occurred at 4 or 5 years of age (58–63). Following the large application of Matacil 1.8D in 1979, the Miramichi blueback herring catch and catch per unit effort in 1984 were the lowest recorded between 1981 and 1987 (Table 5). This coincides with a possible effect of Matacil 1.8D on the spawning process or on survival of eggs or larvae of blueback herring 5 years earlier.

**Discussion**

Our impetus to pursue the possible effects of Matacil 1.8D on fish came from making the connection between reported estrogenic effects concentrations (10 μg/l) of 4-NP in exposures of trout in studies from the United Kingdom (2,69) and knowledge of the formulation and concentrations of aminocarb present in surface waters after operational spraying in New Brunswick in the 1970s and 1980s (1,4). Most current exposure to 4-NP could be expected from effluents where other contaminants and likely higher organic carbon would be present, as opposed to the aerial treatment of forests, with the resulting combination of a carbamate pesticide with 4-NP going into clear forest streams containing minimal solutes. 4-NP was present at 2.6 times the concentration of aminocarb in the Matacil 1.8D formulation. We then looked at possible relationships between application of Matacil 1.8D and the available fisheries data. Atlantic salmon have an extensive fisheries database organized by river system. Our first attempts to link exposure to possible effects on early life stages were unsuccessful. Given the knowledge that smoltification occurs in association with changes in several endocrine systems (8,66), including the levels of reproductive steroids, and Ritter’s comments (31) about the sensitivity of salmon to stress during smoltification, we examined the catch data for effects on the smolt age class. When we applied river-specific knowledge about the predominant age class returning to each river (15W, 25W, or 35W), the relationship between exposure to Matacil 1.8D as smolts and subsequent catch of returning salmon was apparent. This retrospective analysis using recreational catch data for adult Atlantic salmon to assess Matacil 1.8D exposure during a previous freshwater life-stage is possible because salmon home to their native rivers with high fidelity.

Overall, from the 1977 smolt class, the Restigouche River system showed declines in returns of 45% for small salmon and 77% for large salmon. Some of this decline may be due to other factors such as lower sea surface temperature affecting mortality of all smolts at sea, as discussed by Ritter (31). However, despite the overall losses in salmon returns for the 1977 smolt class, there was a significant relationship between the catch for each tributary and how much area was sprayed within that drainage basin. Thus, there was clear evidence of incremental effects dependent on exposure within the larger events of the widespread smolt mortality in 1977. This relationship is apparent using recreational catch data for large fish, which could include 25W and 35W fish. Despite the potential for catch reductions due to Matacil 1.8D application to be compensated by the presence of 35W fish returning to the river, the relationship is still highly significant.

Ritter (31) gave no satisfactory explanation for the high mortality of the 1977 smolt age class. Possible explanations included a lower sea surface temperature. However, if the reductions in catch result from some altered oceanic factor affecting mortality of salmon from all locations, then ranking the salmon assessment areas according to spray exposure should not produce any kind of significant relationship. An association does exist across widely dispersed locations with distinct stocks from the 1977 smolt year class. Although the spray events do not coincide with all of the declines in salmon catch, it is notable that the areas which Ritter (31) lists as unaffected by the high smolt mortality in 1977 occur in regions that were not sprayed (upper Bay of Fundy, lower North Shore of Quebec, Ungava Bay, and northern Labrador) or only received very small exposure (most of the east and south coasts of Newfoundland). The oceanic salmon catch did not decline off west Greenland between 1977 and 1979 (67). Consequently, if there was high mortality of salmon smolts from specific rivers, there could be potential for compensatory reductions in catch on unsprayed rivers. This could add to the apparent area affected by the operational spray program.

The relationships we found for the 1977 spray events were corroborated by looking at independent applications of Matacil 1.8D for other years and in different rivers. The Kolmogorov-Smirnov goodness-of-fit test on these events showed that the predictive power from a spray exposure to indicate years of low salmon catch is nonrandom. The relationship is significant whether or not the spray events of 1977 are included, thereby showing that the effects of other events are independent of the 1977 relationship. The different spray events incorporate data from various areas.
sources, widely separated geographic areas, different jurisdictions of salmon assessment, differing salmon biology, and different jurisdictions for applying and reporting operational forest spraying. Despite this diversity, the relationship between the exposure to Matacil 1.8D and the lower salmon catch was apparent. Thus, exposure to Matacil 1.8D was predictive of when and where effects on salmon might occur.

Our inability to find relationships between the use patterns of another aminocarb formulation (Matacil 1.8F) and another insecticide (fenithrothion) provides some insight to the potential causative factor. If the active insecticide is not responsible, other components of the Matacil 1.8D formulation must then be considered. Based on these observations and the effects of exogenous 4-NP exposure on Atlantic salmon smoltification that Madsen et al. (68) report, we hypothesize that the 4-NP component of Matacil 1.8D is responsible for the observed effects. If forest spraying caused high levels of 4-NP to occur in the environment, this may also provide general insights to a hypothesis about the consequences of exposure to other estrogenic substances common to industrial operations, municipal sewage treatment, and agricultural operations.

The evidence of a coincident population decline in another species suggests that the effects of the forest spraying of Matacil 1.8D could have been fairly broad. Adult spawning, eggs, or larvae of blueback herring represent the possible life stages to be affected, rather than smolts as is the case with Atlantic salmon. Experimental corroboration of the sensitivity of spawning or early life stages of blueback herring to Matacil 1.8D would support the plausibility of this argument. The case for effects on blueback herring in the Miramichi is compelling because commercial catch records indicate that they were present in freshwater during heavy spray applications in 1979. This is potential corroborative evidence in another anadromous species.

Epidemiological Criteria

When monitoring natural populations in relation to contaminant exposure, it can be difficult to establish a causal relationship similar to that derived experimentally. In these instances, the principles of ecoepidemiology are useful in providing a framework upon which to build a balanced judgment (69). We arranged the information about the Matacil 1.8D applications and salmon populations according to seven epidemiological criteria (i.e., probability, time order, strength of association, specificity of association, consistency of association, predictive performance, and coherence) to arrange information about the hypothesis that Matacil 1.8D caused reductions in Atlantic salmon catch. Table 6 summarizes an assessment of how each criterion contributes to our hypothesis. Regression analysis of salmon catch and exposure for the 1977 smolt class in the tributaries of the Restigouche River drainage basin, Spearman rank correlation of the returns of the 1977 smolt class over a broad geographic range, and the Kolmogorov-Smirnov test of salmon catch in different rivers over multiple spray events occurring in different years all proved significant. This suggests a predictive relationship between exposure of salmon smolts to Matacil 1.8D and subsequent declines in catch of adult fish.

In determining causality, an appropriate time order is essential, with the cause preceding the effect. In the rivers we examined, reductions occur after discrete exposure in 16 of the 19 cases, and in 9 of these cases the reductions in catch represented the lowest observed for the entire 11-year period. Thus, the weight of evidence suggests a general compliance with the time order criteria. The fact that some reductions in salmon populations are not specific to Matacil 1.8D exposure simply means that other factors can also contribute to low salmon returns. The degree to which the supposed cause and outcome coincide in their distribution is related to the strength of association. For the rivers examined, where a definite exposure could be assigned, there was a reduction in subsequent catch of returning salmon. This was apparent over broad geographic and temporal distributions of spray events in multiple rivers. Also, the largest reduction in catch is greater in rivers where the population had been exposed than in rivers where the largest reduction in 11 years was not linked to Matacil 1.8D exposure. This, along with the generally similar distribution of exposure and reductions, suggests a good association. In nature it is rare that any effect is solely associated with a particular cause. This proves true in our situation, where reductions in salmon catch may also be a result of overfishing, disease, habitat degradation, predators, environmental conditions at sea, availability of food, etc. The lack of specificity of cause for our observed response (reduction in catch) is not uncommon and does not detract from our inference of causality.

The observed reductions in salmon catches have been recorded by different investigators, in different locations, and at different times following Matacil 1.8D spray events. This and reductions in blueback herring catch also coinciding with heavy Matacil 1.8D applications to the Miramichi in 1979 suggest a consistency of association, although the life-stage affected in blueback herring may differ. The plausibility of our hypothesis is consistent with the weight of evidence and does not conflict with general known facts, natural history, and biology of Atlantic salmon or the possible responses to Matacil 1.8D. Although experimental testing determining the effects of Matacil 1.8D on smolt survival have yet to be completed, our evidence implies that the 4-NP component of Matacil 1.8D may affect the smolting process and influence survival of the smolt- ing age class. The exposure–response relationship between area sprayed and salmon catches for the 1977 smolt class in the tributaries of the Restigouche River system also suggests a strong coherence.

| Table 6. Epidemiological criteria* | Evidence                          |
|----------------------------------|-----------------------------------|
| Criterion                        | Effect on hypothesis          |                                |
| Probability                      | ++                               | Nonparametric tests significant |
| Time order                       | ++                               | Weight of evidence support     |
| Strength of association          | ++                               | The effect is distributional    |
| Specificity of association       | ?                                | Needs experimental verification|
| Of effect                        |                                  |                                 |
| Of cause                         | ?                                | Catch declines can be associated|
| Consistency of association       |                                  | with multiple causes           |
| Different investigators          | +++                              | Salmon catch reductions        |
| Different locations              | +++                              | Salmon catch reductions        |
| Different times                  | +++                              | Salmon catch reductions        |
| Different species                | +                                | Blueback herring catch reductions|
| Predictive performance           | ++                               | Locations and times of the effect|
|                                  |                                  | are predictable                 |
| Coherence                        | Theoretical                      | Plausible                       |
|                                  | Factual                          | Compatible                     |
|                                  | Biological                       | Plausible                       |
|                                  | Dose response                    | 1977 smolt class in Restigouche tributaries |

Abbreviations: ++++, strong association; ++, moderate association; ?, association unclear.

*Criteria from Fox (68).
Potential mechanism of effect. The discussion of potential mechanisms is limited by our current lack of experimental data about the effects of Matacil 1.8D on salmon smolts. However, some speculation about plausible mechanisms is essential to the development of testable hypotheses. Smolitification is a highly demanding time for young salmon and additional stressors could prove serious, as the process of moving to saltwater and subsequent survival is both energetically and metabolically challenging (8.9,31). Smolitification is a complex developmental process occurring in association with changes in the activity of several hormonal systems, including reproductive steroids, that facilitate the physiological processes necessary for sea water acclimation (66,70–72) as well as home stream memory (10,73). Thus, effects of Matacil 1.8D could range from an altered chemical perception of home stream odor to direct or indirect effects on smolt growth or hypo-osmoregulatory ability.

Aminocarb is a broad-spectrum carbamate insecticide that exerts its toxic effect by inhibiting cholinesterase enzymes. The LC₅₀ (concentration required for 50% mortality) of waterborne aminocarb to juvenile Atlantic salmon (Salmo salar) was found to be 8,700 µg/l in a static test (74). During forest spray operations, mean concentrations of aminocarb in flowing water were well below the LC₅₀, reaching 10 µg/l within 4 hr of spraying, while peak concentrations measured in standing water were up to 331 µg/l (Table 7) (75). According to whole body fish tissue analysis, there is little accumulation of aminocarb in fish after operational forest spraying (76–78). Due to its low log Kᵢₑ (1.85) and its rapid dissipation, aminocarb would not be expected to accumulate efficiently in fish (4). Aminocarb concentrations were probably not high enough to produce effects. This is consistent with finding no association between reduced salmon catch and use of the Matacil 1.8F formulation that did not contain 4-NP.

4-NP is a part of the basic molecular structure of a family of compounds called alkylphenol polyethoxylates (APECO), which are surfactants in use throughout the world for a variety of purposes (79). 4-NP and some closely related 4-nonylphenol ethoxylates and carbamates are found in effluents from secondary treatment facilities and municipal sewage treatment plants (79–81). 4-NP is bioaccumulative once in the aquatic environment (3,7,82). The LC₅₀ of 4-NP to Atlantic salmon juveniles was 900 µg/l in a static test (7) and 130 µg/l in a 96-hr flow-through test (76). Because Matacil 1.8D contained 4-NP as a primary solvent, at 2.6 times the weight of the active ingredient aminocarb, it is not unreasonable to assume that environmental concentrations of 4-NP could be 2.6 times higher than the concentrations reported for aminocarb. Ernst et al. (83) measured both aminocarb and 4-NP in three water samples from a lake in northern New Brunswick and found 4-NP concentrations were over 3 times the concentration of aminocarb in all samples. Given the reported aminocarb levels (see Table 7), environmental levels of 4-NP would be expected to reach at least 20 µg/l within 4 hr of application and show peak levels in standing water of >800 µg/l. Holmes and Kingsbury (6) report on a single simulated operational spray of 4-NP, without aminocarb, at the maximal annual dosage. They found a 4-NP concentration of 9 µg/l present in running water 1 hr after spray, which declined to 3 µg/l after 4 hr. The concentration of 4-NP was 1,100 µg/l in standing water behind a beaver dam at 4 hr after spray, and was still 12 µg/l the next day. Chronic waterborne exposures of 4-NP in the 1–20 µg/l range produced effects on growth (84) and induced vitellogenin production (3) in salmonids. Waterborne 4-NP is rapidly taken up and distributed throughout the body in salmonids (85), and studies by McGlense et al. (7) show a nonequilibrium factor of 175× for a 1-day exposure. Thus, expected tissue concentrations of 4-NP in salmon could be as high as 3.5 mg/kg given waterborne exposures of 20 µg/l 4-NP (see Table 7). Although considered weak estrogenic, the ED₅₀ effective dose required for the desired end point) for estrogenic potency of 4-NP of about 16 µM (2) in salmonid hepatocytes is also equivalent to 3.5 mg 4-NP/kg tissue. Thus, it seems plausible that the aerial spray of Matacil 1.8D produced levels of 4-NP sufficient to initiate estrogenic responses in exposed salmonid.

The known antagonistic effects of exogenous gonadal steroids on smolitification may represent one mechanism whereby xenosterogens may influence subsequent returns of salmon. Recently, several studies (86–91) showed that smolitification and sea water adaptability are impaired by exogenous treatments with reproductive steroids. While there was a difference in relative potency, Madsen et al. (68) also showed that 4-NP and 17β-estradiol exhibit qualitatively similar inhibitory effects on smolitification and

Table 7. Concentrations of aminocarb measured in water samples collected inside and outside treated areas* and calculated nonylphenol concentrations b

| Sampling delay (hr) | Lotic environments (µg/l) | Lentic environments (µg/l) |
|--------------------|---------------------------|---------------------------|
|                    | n | Mean ± SD | Median | Range | n | Mean ± SD | Median | Range |
| **Inside treated area** | | | | | | | | |
| Aminocarb (measured) | | | | | | | | |
| 1–4                | 9 | 10.0 ± 9.4 | 8.63 | 0.1–30.5 | 7 | 67.2 ± 116.9 | 25.0 | 7.95–331.0 |
| 5–24               | 6 | 6.6 ± 13.5 | 0.999 | 0.18–34.0 | 5 | 18.8 ± 35.0 | 1.99 | 0.11–81 |
| >24                | 3 | 0.08 ± 0.02 | 0.075 | 0.066–0.61 | 3 | 48.7 ± 91.0 | 5.174 | 0.286–210.6 |
| Nonylphenol (calculated) | | | | | | | | |
| 1–4                | 9 | 18.9 ± 24.4 | 22.44 | 0.26–79.3 | 7 | 174.7 ± 303.9 | 65.0 | 20.67–860.6 |
| 5–24               | 6 | 17.2 ± 35.0 | 20.2 | 0.468–88.4 | 5 | 48.7 ± 91.0 | 5.174 | 0.286–210.6 |
| >24                | 3 | 0.2 ± 0.05 | 0.2 | 0.1716–0.26 | 3 | 48.7 ± 91.0 | 5.174 | 0.286–210.6 |
| **Outside treated area** | | | | | | | | |
| Aminocarb (measured) | | | | | | | | |
| 1–4                | 1 | 13.5 | 13.5 | | 2 | 0.9 ± 1.1 | 0.877 | 0.133–1.62 |
| 5–24               | 2 | 1.1 ± 1.5 | 1.062 | 0.013–2.11 | 1 | 0.9 | 0.9 | |
| >24                | 3 | 0.03 ± 0.04 | 0.01 | 0.01–0.071 | 1 | 2.3 ± 2.7 | 2.279 | 0.346–4.212 |
| Nonylphenol (calculated) | | | | | | | | |
| 1–4                | 1 | 35.1 | 35.1 | | 2 | 2.3 ± 2.7 | 2.279 | 0.346–4.212 |
| 5–24               | 2 | 2.8 ± 3.9 | 2.76 | 0.034–5.486 | 1 | 2.34 | 2.34 | |
| >24                | 3 | 0.08 ± 0.09 | 0.01 | 0.01–0.071 | 1 | 2.34 | 2.34 | |

SD, standard deviation.

*From Marotte et al. (78).

*bNonylphenol concentrations were calculated by multiplying the aminocarb concentrations of Marotte et al. (78) by a factor of 2.6; the ratio of nonylphenol to aminocarb in Matacil 1.8D formulation. This is considered a conservative estimate because Ernst et al. (83) found that on average the ratio of aminocarb to nonylphenol in environmental samples is 3.2±1.
hypo-osmoregulatory physiology of Atlantic salmon. Differences between these laboratory studies and fish exposed via the aerial spray program include amount, route, and relative duration of exposure. In the previously described laboratory studies, exogenous steroid and 4-NP treatments were by injection, implants, or dietary exposure to high doses. The inhibitory effects of environmentally representative waterborne concentrations of 4-NP on smoltification and the hypo-osmoregulatory physiology of Atlantic salmon awaits experimental demonstration. It may be significant, however, that mortality from 43 to 60% was reported in two studies in which smolts were exposed to reproductive steroids and subsequently given a sea water challenge (69,91). Other organisms undergoing major developmental or metamorphic processes may also be at risk. Gonadal hormones have been shown to inhibit the induction of metamorphosis in an amphibia tadpoles in vivo (92), and field study with operational Matacil showed retarded development in exposed tadpoles (93).

This paper is significant because it demonstrates a relationship between chemical use and salmon catch, which could potentially affect the sustainability of the salmon fishery. While the study provides epidemiological evidence that past use of Matacil 1.8D was related to lower abundance of Atlantic salmon, mediating mechanisms should be determined. This study also develops broader questions about possible impacts on present day stocks of anadromous fish that deserve further attention. Although Matacil 1.8D is no longer used, recent papers have speculated that 4-NP could have effects on fish in the environment under current exposure patterns (3,65,68). The estimated levels of 4-NP present after forest spraying fall in the same range as those currently found in pulp mill discharges, industrial effluents, and municipal sewage outfalls (79). If the effects exerted by Matacil 1.8D are due to the estrogenic potential of the 4-NP formulation, then estrogenic activity stemming from other sources, i.e., domestic sewage, agricultural waste, or phytoestrogens from pulp mills, might also influence present day salmon populations.

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