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EFFECTS OF TFM AND BAYER 73 ON GILL ULTRASTRUCTURE OF SEA LAMPREY AMMOCOETES AND RAINBOW TROUT FRY

by

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Sea lamprey control efforts rely on the use of lampricides, primarily TFM (3-trifluormethyl-4-nitrophenol), and secondarily Bayer 73 (5,2´-dichloro-4´-nitrosalicylanilide). If more detailed information were available on how these compounds affect sea lamprey ammocoetes (Petromyzon marinus), it might be possible to identify other compounds that are more effective or more selective. This would be of extreme value if the use of TFM is ever curtailed or eliminated. Furthermore, knowledge of the modes of toxicity of these lampricides could lead to the development of synergist chemicals, which when applied with TFM, could enhance toxicity of this compound, further exploiting lampreys´ special vulnerabilities.

As its primary mode of toxicity, TFM has been proposed to inhibit cell respiratory metabolism (Kawatski and McDonald 1974; Howell et al. 1980). TFM is said to uncouple oxidative phosphorylation from electron transport, yet

experiments have not demonstrated the stimulation of cellular oxygen consumption rate that such a hypothesis requires (Kawatski and McDonald 1974). Alternatively, many compounds poisonous to fish kill primarily by disrupting gill function, and this has been suggested for TFM (Christie and Battle 1963; Youson and Freeman 1976). This possibility was investigated in the current study.

Also of interest is the mode of selectivity of TFM and Bayer 73. Teleosts are less susceptible to these compounds than are lampreys, and we investigated whether this is reflected in the way the lampricides affect the gills. Gills of rainbow trout fry (Salmo gairdneri) exposed to TFM and Bayer 73 were viewed along with gills of sea lamprey ammocoetes exposed under similar conditions.

MATERIALS AND METHODS

Two series of lampricide exposures were performed, one in Michigan at the Hammond Bay Biological Station, using Lake Huron water, and another at the laboratory of the principal investigator in Pullman, Washington, using dechlorinated local tap water. For the Michigan exposures, trout fry came from local hatcheries, lamprey came from local streams, and all had been held several months as a part of the Hammond Bay Station's stock of experimental animals. For the Washington exposure series, the trout fry had been hatched and raised by Dr. Gary Thorgaard and Mr. Paul Sheerer at facilities at Washington State University, and the sea

lamprey larvae had been obtained from the Marquette Biological Station of the U.S. Fish and Wildlife Service, then held in our laboratory for several months (Mallatt, 1983). Ammocoetes and trout fry were of roughly similar sizes: ammocoetes ranged from 0.3 to 1.2 g and trout ranged from 0.7 to 3 g, wet weights.

Exposure vessels were glass aquaria containing 10 to 15 liters of water. Groups of 3 to 10 animals were exposed in each vessel, and there was less than 0.75 g of fish per liter of exposure water. Ammonium and nitrate levels in the exposure water remained at undetectable levels throughout the exposure periods. The water was aerated with airstones. Exposure temperature was 11-13 ^{o}C. The Hammond Bay animals were brought to the 13^{o} exposure temperature from 18^{o} at the rate of 2^{o} per day just prior to the test.

In Michigan, the water was of pH 7.95-8.1, total hardness of 130 mg $CaCO_3$/l, and total alkalinity of 90 mg $CaCO_3$/l. In Washington, water parameters were pH 8.35, total hardness 135, and alkalinity 150. Note the two waters differed in alkalinity.

Groups of ammocoetes and trout fry were exposed to static (non-renewed) solutions of TFM, Bayer, and TFM/Bayer, at various toxicant concentrations, for periods of 2.5 to 12 hours. Exposure conditions are listed in detail in Table 1. The primary goal was to mimic the lampricide treatments in streams. Thus, most exposures involved lampricide concentrations that would produce 100% mortality of sea

lamprey ammocoetes within 9 hours, i.e., the lamprey 9 h LC100 (Table 1, Part I). Trout fry, besides being exposed to concentrations of lampricides lethal to lampreys, were also exposed to the higher concentration of TFM that was lethal to trout, i.e., the trout 9 h LC100 (Table 1, Part II). Finally, we exposed some lampreys for 12 hours to their 96 h LC50 level of TFM and Bayer (Table 1, Part III), as this is a standard concentration used in many toxicological studies. The reason that the tests were split between Washington and Michigan is given below.

Experiments began as the animals were placed in the lampricide solutions. Toxicant concentrations, as measured colorometrically and by gas chromatography, stayed constant (±10%) throughout the exposure periods. In exposures where mortality occurred, animals began to be removed as soon as some neared death, and animals continued to be removed until the last remaining one was moribund. Ammocoetes and trout fry were classified as either `well´,` sick´, or `dead´. `Well´ ammocoetes and trout reacted to light touch with a probe by swimming away. `Sick´ ammocoetes were semi-paralyzed, unable to swim and only able to bend their trunks in response to probing. `Sick´ trout gasped for air at the water surface and had lost equilibrium while swimming. Sick animals, especially the trout, were obviously near death, and would die within minutes if not removed. Death was defined as lack of pharyngeal ventilatory movements and failure to respond to prodding.

When taken from the exposure vessels, ammocoetes were anesthetized for ten minutes in tricaine methanesulphonate solution (0.1 g/l). This was done because unanesthetized ammocoetes squirm and are difficult to handle---and they can not be quieted by a blow to the head. Studies in our laboratory (Mallatt, unpublished) indicate that anesthetization does not affect normal ammocoete gill morphology. Trout fry removed from the exposure vessels were not anesthetized but were stunned by a blow to the head (a few control trout were anesthetized and their gills did not differ from gills of un-anesthetized trout). Next, in lampreys, the pharynx was opened by a transverse cut through the region of the third gill, and the pharynx was immersed in fixative solution. In trout, a single gill was dissected from the pharynx (first gill, left side) and immersed in fixative, in a process that took less than two minutes. Fixation was at ambient temperature (11-13^{o}), and the fixative was 4% paraformaldehyde - 1% glutaraldehyde in 0.1M phosphate buffer. The gills were processed for transmission electron microscopy and for high resolution light microscopy by routine methods, which are presented in detail by Mallatt and Ridgway (1984). All sections were taken perpendicular to the gill respiratory lamellae. Two lamellae were examined per animal by electron microscopy.

RESULTS

General Considerations

The normal morphology of ammocoete and trout gill respiratory lamellae will be briefly described (Figs. 1-3; Figs. 18-19). These lamellae consist of an outer epithelium and an inner blood vessel called the lamellar blood sinus (Morgan and Tovell 1973; Mallatt and Ridgway 1984). The epithelium is two cell layers thick (Figs. 1, 18). The deeper of these two layers consists of basal cells, which show features indicative of an undifferentiated state (high nucleus to cytoplasm ratio, many ribosomes, poorly developed endoplasmic reticulum). Basal cells presumedly give rise to the more superficial cell layer of the lamellar epithelium. These superficial epithelial cells are of two types: in lampreys (Figs. 2, 3), one is a cell rich in large mitochondria, thought to be involved in the reclamation of sodium and chloride ions lost from the animal to the ambient fresh water through diffusion (Morris and Pickering 1975); this is the presumed ion-uptake cell. The other superficial cell type on the ammocoete gill lamellae is the mucous-platelet cell, which looks much like a basal cell except that it contains small apical mucous secretory granules and a better developed rough endoplasmic reticulum (RER). Mucous-platelet cells occur mostly near the lamellar tip, and are thin, for respiratory exchange. Some white blood cells (lymphocytes) occur within the lamellar epithelium (Fig. 3). Deep to the epithelium, the lamellar blood sinus is lined and spanned by barrel-shaped pillar cells.

In trout gill lamellae (Figs. 18, 19), the two superficial epithelial cell types are called pavement cells and chloride cells. They correspond to the mucous-platelet and ion-uptake cells, respectively, of ammocoete gills. Trout chloride cells show the large mitochondria of ammocoete ion-uptake cells, but they also contain a complex system of cytoplasmic tubules; such tubules are absent from ammocoete ion-uptake cells. Trout lamellar blood sinuses also contain pillar cells.

Gills from the unexposed control animals in our study generally exhibited normal morphology. Lamellae from one of the nine control ammocoetes showed some breakage of organelles within cells of the lamellar epithelium. Our experience with lamprey gills in general indicates that a small percentage of control animals typically show such damage.

Fixation was optimal for the lamprey gills, but it proved difficult to obtain good preservation of trout gills. The trout gills from the Michigan exposures showed fixation artifacts in the form of extremely large intercellular spaces in the gill lamellar epithelium, and broken epithelial cell membranes. The experiments employing trout fry were repeated four times in Michigan, using a variety of fixatives, in an effort to eliminate these artifacts, but the efforts proved unsuccessful. This is why the definitive trout exposures were done in Washington, where good fixation of trout gills was readily achieved. (We employed some

ammocoetes in Washington, as well as in Michigan, to assure the effects of lampricides on ammocoete gills were the same in both places.)

Effects of lampricides on ammocoete and trout gills

Lampreys

1. TFM: 2.5 mg/l in Michigan, 2.5 to 6 hour exposures

Gills of sea lamprey ammocoetes showed abnormalities after exposure to this 9 h LC100 of TFM (Figs. 4, 5). Gills from the `well´ ammocoetes did not seem to differ from gills of `sick´ individuals, so they are described together. The respiratory lamellae thickened (Fig. 4), partly due to the appearance of widened intercellular spaces in the epithelium, and partly due to swelling of the most abundant epithelial cell type, ion-uptake cells.

At the cellular level, almost all damage was confined to ion-uptake cells alone. Abnormal features in these cells included (Fig. 5) loss of surface microvilli, frequent presence of clear vacuoles in the cytoplasm, and enlarged mitochondrial profiles. Occasionally, the Golgi complex was disorganized and vacuolated, and mitochondrial cristae had lost their regular parallel arrangement to take on a more random arrangement. In extreme cases, these cristae were swollen. The ion-uptake cells of TFM-exposed lamellae occurred about as frequently as in the control gill lamellae, comprising about 75% of the superficial cells. Very few ion-uptake cells had proceeded to the point of cell

death (or necrosis, defined as extreme cell swelling and loss of cytoplasmic electron density, plus shattered organelles).

In six of the eight aammocoetes examined, basal and mucous-platelet cells appeared normal. In two animals, however, basal and mucous-platelet cells showed some abnormality. The nuclear profiles and cytosol were lighter than normal. Some such cells showed swollen and broken mitochondria and RER. These features pre-sage abnormalities more commonly seen in lamprey gills exposed to Bayer 73 (see below).

No other cell type in the TFM-exposed gill lamellae showed any abnormalities. Pillar cells were unaffected, as were the white blood cells (lymphocytes) in the lamellar epithelium. Our light microscope sections indicated that goblet cells on the lateral sides of the gill filaments were normal, with a full complement of mucous secretory granules. Muscle, arteries, and skin all appeared normal.

2. TFM: 5 mg/l in Washington, 6 - 9 hour exposures

Ammocoetes exposed to this lethal concentration of TFM in our laboratory in Washington (Fig. 6), exhibited similar, but noticeably milder, gill damage than occurred in ammocoetes exposed to half this concentration in Michigan. Mild hypertrophy of some ion-uptake cells and of their mitochondria were evident. The mitochondrial cristae of such cells were not disoriented, and microvilli remained intact on the surface of most ion-uptake cells.

3. TFM: 1.6 mg/l in Michigan, 12 hour exposures

Gill lamellae of ammocoetes exposed to this 96 h LC50 appeared normal except for the presence of small, clear vacuoles in a few ion-uptake cells (Fig. 7).

4. Bayer 73: 55 ug/l, in Michigan, 5-7.5 hour exposures

This lethal concentration of Bayer 73 was associated with abnormalities in all type of epithelial cells on ammocoete gill lamellae. However, the lamellae generally retained their normal thin finger-like appearance in section (Fig. 8), and did not thicken like gill lamellae exposed to TFM. The epithelium of Bayer-exposed lamellae tended not to be thickened, because . . . 1) this epithelium generally contained no widened intercellular spaces; 2) because hypertrophy (swelling) of ion-uptake cells was not as extreme as in TFM poisoning; and 3) because ion-uptake cells were rarer on Bayer-treated gill lamellae (only 40% of all superficial cells), leaving the thin mucous-platelet cells to cover most of the lamellae.

Despite the differences in the overall appearances of the Bayer vs. TFM-exposed lamellae, the ion-uptake cells of Bayer-exposed gills show the same kinds of alterations as in TFM-exposed gills (Fig. 9). Though the degree of hypertrophy is less, ion-uptake cells show loss of microvilli, big clear vacuoles, vacuolation of the Golgi apparatus, and scattering and whorling of Golgi cisterns. Some mitochondrial profiles were enlarged, and contained disoriented cristae.

Bayer exposure was associated with necrosis of some ion-uptake cells (Fig. 10). Necrotic ion-uptake cells showed very lucent cytoplasmic, and mitochondrial matrices, broken cell membranes, and extreme swelling of the RER and nuclear envelope. Many such ion-uptake cells had desquamated (i.e., detached) and were seen free in the spaces between gill lamellae.

Abnormalities in basal and mucous-platelet cells were widely evident in Bayer-exposed gill lamellae (Figs. 10-13). Such cells were most affected near the tips of the respiratory lamellae. Similar kinds of changes occurred in both basal and mucous-platelet cells. The least altered of these cells exhibited a lightening and enlargement of the nucleus (Figs. 8, 11). The cytosol lightens too, as if taking up fluid. Organelles, which are normally closely packed within basal cells, spread apart and become more distinctly visible. In the next stage of cell alteration (Fig. 12), some mitochondria are swollen and broken, and the cristae in other mitochondria are swollen. Large, clear vacuoles appear in the cytoplasm of some basal and mucous-platelet cells. The Golgi apparatus is broken or vacuolated, and the RER and nuclear membrane swell. This stage grades into necrotic basal and mucous-platelet cells, with damage to all membrane-lined organelles, and a white cytosol. The mucous secretory granules of mucous-platelet cells are lost by the necrotic stage, but not before this.

Epithelial lymphocytes (not illustrated) always showed less damage than did any other cell type in the Bayer-exposed lamellar epithelium. In these lymphocytes, the cytoplasm did not swell or lighten. A few clear cytoplasmic vacuoles occasionally were seen, and mitochondrial profiles may have been slightly enlarged in these lymphocytes. Pillar cells in the lamellar blood sinus remained normal.

Paradoxically, in these Bayer 73 exposures, ammocoetes that were sacrificed when well (Fig. 13) exhibited greater average damage to the gill lamellae than did ammocoetes sacrificed when near death (sick: Fig. 8). For example, two of five `well´ ammocoetes showed almost complete necrosis of the lamellar epithelial cells, but no `sick´ animals showed such extensive cell death. The paradox may be explained by the fact that `well´ animals averaged a longer exposure to the Bayer solution (5.5 - 7 h) than did the `sick´ individuals (5 - 6 h).

Light microscopic observation indicated that the goblet cells on the lateral sides of the gill filaments and on the side wall of the pharynx were affected by Bayer. While goblet cells seemed less altered than nearby basal and mucous-platelet cells, some goblet cells had the RER dispersed into several large pieces. The apical mucous secretory granules were exhausted in many goblet cells, hinting that Bayer, unlike TFM, stimulates branchial mucous secretion. There was no goblet cell necrosis. Some goblet cells showed clear vacuoles in the apical cytoplasm.

There seemed to be no Bayer-induced damage to other epithelial types in the ammocoete pharynx (ciliated, protective: see Mallatt and Ridgway 1984), at least none was detectable with light microscopy.

To summarize the effects of Bayer on ammocoete gills, we conclude that like TFM it preferentially affects ion-uptake cells. The effects of Bayer, however, are more severe than those of TFM. Bayer caused more necrosis of ion-uptake cells, and it more commonly led to changes in several other gill cell types: mucous-platelet, basal, and goblet cells.

One further thing should be noted about the Bayer exposures. Bayer 73 is a low solubility compound that must be delivered to the exposure water via a carrier solvent, dimethylformamide (DMF). The concentration of carrier, following standard protocol at the Hammond Bay Biological Station, reached 1.7 ml per liter of exposure water. Dimethylformamide is not highly toxic, but the concentrations used considerably exceed the levels recommended by the U.S. Environmental Protection Agency (0.5 ml/l for acute studies: Stratton 1985). This concentration of DMF conceivably could have induced artifactual changes in the Bayer-exposed gill lamellae of lampreys. Investigation is currently under way at Washington State University to determine if DMF affects lamprey gill structure.

5. Bayer 73: 36 ug/l in Michigan, 12 hours exposure

Gills of ammocoetes exposed to this 96 h LC50 level of Bayer were examined with light microscopy only (not

illustrated). Nonetheless, it was clear that the gills were affected. Some ion-uptake cells were necrotic and desquamating in gills of four of the five ammocoetes examined; however, ion-uptake cell death was not as frequent as in the lethal Bayer exposures described above. In basal and mucous-platelet cells of the gill lamellae, the nuclei did not appear to be lightened, suggesting these cells were not affected. Goblet cells appeared normal.

The 96 h LC50 of Bayer clearly affected gill structure, while the corresponding concentration of TFM had almost no effect (see above). This is further evidence that Bayer affects ammocoete gill structure more severely than does TFM, given comparable exposure conditions.

6. TFM/Bayer Mixture: 1.25 mg TFM and 20 ug Bayer/l, in Michigan, 4 - 7.5 hour exposures

Gill lamellae of ammocoetes exposed to this lethal mixture of TFM and Bayer (the 9 h LC100) showed an interesting combination of the effects of both lampricides, with those effects that are shared by the two toxicants being markedly intensified.

In their overall appearance, the lamellae ranged from normal thickness (Fig. 14) to being slightly widened. About 50% of the lamellar surface was covered by ion-uptake cells (Fig. 14), and 50% by mucous-platelet cells, which is intermediate between the condition in TFM-exposed lamellae (75% ion-uptake cover) and Bayer-exposed lamellae (40% ion-uptake cover). Widened intercellular spaces were not common

in the lamellar epithelium, resembling gills exposed to Bayer alone in this respect. Gills from `well´ and `sick´ ammocoetes showed similar degrees of damage.

In ion-uptake cells (Fig. 15), the cytological changes are those seen in gills treated with TFM or Bayer alone. (That is not surprising, as the effects of these two lampricides on ion-uptake cells are qualitatively identical anyway.) Alterations include hypertrophy of the whole cell and of the mitochondria, disorientation of mitochondrial cristae, loss of microvilli, presence of clear vacuoles, and vacuolation of the Golgi apparatus.

The TFM/Bayer mixture produces an exaggerated number of dead ion-uptake cells (Fig. 14). Many more necrotic and desquamating ion-uptake cells occur here than in gills exposed to Bayer alone. Just below those ion-uptake cells that are in the process of leaving the epithelium are clusters of clear vacuoles in the cells left behind (Fig. 16).

Many basal and mucous-platelet cells exhibit the abnormalities seen earlier to be induced by Bayer alone: lightening and swelling of the nucleus and the cytosol (Fig. 17). A few such cells had proceeded to the stage where some mitochondria and the Golgi apparatus were broken and microvilli were lost; however, basal and mucous-platelet cells rarely reached the necrotic stage. The TFM/Bayer mixture (Bayer = 20 ug/l) did not affect mucous-platelet and

basal cells as severely as did the lethal concentration of Bayer alone (55 ug/l).

Wide vacuoles were occasionally observed in the junctional complexes between the surface epithelial cells, as if these junctions were pulling apart (Fig. 16).

Epithelial lymphocytes and the pillar cells of the lamellar blood sinus retained normal morphology. A very rare type of cell occurs in the epithelium between the lamellae in ammocoetes, the `true chloride cell´ (Mallatt and Ridgway 1984). Such cells were seen often enough in these TFM/Bayer sections to determine their morphology; they appeared fully normal.

As judged by light microscopy, all other epithelial cell types in the TFM/Bayer ammocoete pharynx appeared normal, except the goblet cells. These showed the typical effects of Bayer (RER in several pieces, secretory granules exhausted from many goblet cells).

To summarize the effects of TFM/Bayer, the mixture combined the effects of both toxicants, and produced an exaggerated effect on ion-uptake cells, resulting in extensive necrosis of these cells.

7. TFM/Bayer 73 mixture: 1.9 mg TFM and 23.75 ug Bayer/l, in Washington, 7 hour exposure.

A single ammocoete was exposed to this lethal mixture in Washington, to confirm the results of the Michigan experiments. Effects on the gills were indeed the same, and of the same intensity.

Trout

Rainbow trout fry exposed to a lethal concentration of TFM, 30 mg/l for 9 hours in Washington, retained a fully normal gill morphology (Figs. 20-22). The same held for trout exposed for 9 hours to 5 mg/l TFM and for trout exposed for 9 hours to a TFM/Bayer mixture lethal to ammocoetes (the lamprey 9 hour LC100: 1.9 mg TFM, 23.75 ug Bayer/liter). Fixation was good in this Washington trout tissue, and pavement, basal, chloride, and pillar cells of the gill lamellae all exhibited the same normal ultrastructure seen in control trout gills.

As explained above (Table 1), the definitive, Washington, experiment on trout did not include any exposure to Bayer 73 alone. However, one trout specimen exposed in Michigan to 55 ug Bayer/l was examined and it showed nearly acceptable fixation (Fig. 23); the lamellae looked normal, like those of the best-fixed Michigan control trout specimens.

In conclusion, no effects of lampricide could be identified on trout gills.

DISCUSSION

Summary of Main Findings of the Study

1. Lethal concentrations of TFM, Bayer 73, and a TFM/Bayer mixture, markedly affected the gill morphology of sea lamprey ammocoetes. By contrast, a lethal concentration of

TFM had no effect on the gill structure of Rainbow trout fry, nor were trout gills affected by a TFM/Bayer mixture (the lamprey LC100).

2. TFM almost exclusively affected a single gill cell type in ammocoetes, the ion-uptake cell; swelling and vacuolation of this cell type occurred upon TFM exposure, but not much necrosis.

3. TFM produced widened intercellular spaces within the ammocoete gill epithelium; Bayer and the TFM/Bayer mixture did not.

4. Pullman Washington water has a higher alkalinity than does the Lake Huron water used at the Hammond Bay Biological Station. In the more alkaline Pullman water, lethality of TFM to ammocoetes was reduced (higher LC100 value), and the degree of gill damage produced by a lethal dose of TFM was also less.

5. Bayer 73 affects the structure of ion-uptake cells of ammocoetes in essentially the same way as TFM does, but Bayer produces more necrosis of this cell type.

6. Bayer, much more than TFM, affects other gill epithelial cell types in ammocoetes: basal and mucous-platelet cells. Bayer exposure results in lowered electron density of

cytoplasm and nucleus, in organelle damage, and in necrosis of these cells.

7. Bayer 73, unlike TFM, may affect the gill goblet cells of ammocoetes, inducing mucous hypersecretion. (This conclusion is tentative, however, being based only on light microscopy.)

8. Overall, Bayer results in more damage to the ammocoete gill than does a comparable concentration of TFM.

9. A lethal TFM/Bayer mixture produces both the effects of TFM and the effects of Bayer on the ammocoete gill, although the incidence of necrotic ion-uptake cells is exaggerated.

10. In all the lampricide exposures in this study, the epithelial cells of the gill lamellae were more affected by lampricide than were any other cells, tissues, or organs that were evident in our histological sections through the anterior body of ammocoetes (e.g., skin, muscle, blood vessels, other pharyngeal epithelium).

What Results Say About Mode of Toxicity of Lampricides

Lethal concentrations of TFM and of Bayer 73 caused marked gill alterations in sea lamprey ammocoetes, and a lethal mixture of the two lampricides further magnified the gill damage. Organs outside the gill, as seen in our

histological sections, were not obviously affected. This raises the possibility that gill lamellae are the target organs of lampricides, and that damage to gills is the primary cause of mortality of ammocoetes exposed to these chemicals. This is a simple theory that can be tested directly. Given the alterations in the ion-uptake cells, which are presumedly involved in ion regulation, blood NaCl concentration is expected to drop in ammocoetes exposed to TFM or Bayer. Similarly, the gill damage could disrupt respiratory exchange, so blood oxygen tension should be lowered during lampricide exposure (but see Agris 1967). The above measurements are worth making, in further experiments.

A more conservative hypothesis is that gill damage is not the primary cause of death, but merely contributes to the stress of lampreys exposed to TFM. Inhibition of cell respiratory metabolism throughout the body might still prove to be the primary toxic effect (Howell et al. 1980), with gill disruption leading to secondary symptoms. Even so, the gill damage is likely to aggravate any primary effects and therefore accelerate mortality. Thus, capitalizing on the tendency of lampricides to damage lamprey gills could be a way of increasing the efficacy of of these toxicants.

Mallatt et al. (1985) have recently documented the ultrastructural damage to gills of sea lamprey ammocoetes produced by a sublethal exposure to TFM. Animals in that study were exposed to 2.25 mg TFM/l for nine hours (in

Michigan), compared to 2.5 mg/l for 2 - 6 hours in the present study. In the previous study, damage to gill lamellae was qualitatively similar to that recorded here, but paradoxically, the alterations in the ion-uptake cells were more extreme. For example, more ion-uptake cells reached advanced stages of damage and necrosis in the previous study. Apparently, the shorter exposure period of the present study explains the milder degree of damage, overriding any effects of the slightly higher TFM concentration employed.

TFM targets gill ion-uptake cells and in turn may interfere with the ability of ammocoetes to maintain normal concentrations of Na+, Cl-, and other ions in their blood and body fluids. The hypothesis that ion loss contributes to death in TFM-poisoned ammocoetes is supported by the finding that ammocoetes are more susceptible to TFM in soft water than in hard water (Howell et al. 1980). High ambient calcium ion concentrations, as are present in hard waters, can slow the rate at which sodium and chloride ions leak from fish gills under certain conditions (McDonald 1983).

Mallatt et al. (1985) suggested ammocoete ion-uptake cells, like similar gill cells in other fish, are able to remove xenobiotic compounds from the blood and expel them from the gills. Organic acids are especially likely to be concentrated by such cells (Motais and Romeu 1972), and TFM is an organic acid. Concentrated in the ion-uptake cells,

the lampricide would exert its toxicity there, explaining the preferential damage to this cell type by TFM.

A new and intriguing finding of the present study is that Bayer 73 produced alterations in the ammocoete gill that are similar, and even more severe, than the effects of TFM. The nitrosalicylanilide molecule is similar to the nitrophenol molecule in some respects (phenol ring with OH and NO_2 groups, attached halogen atom), so it is perhaps not surprising that the anatomical effects induced by the two compounds on ion-uptake cells are similar. Bayer 73, however, seems to be even more cytotoxic to these cells than is TFM.

Bayer 73 affected basal and mucous-platelet cells in the ammocoete gill lamellae. Unlike ion-uptake cells, these cells are not thought to concentrate toxicants, so the effect of the lampricide on these cells must be direct. It is not clear why basal and mucous-platelet cells should show more Bayer-induced damage than most other cell types in the lamprey body. Functionally, basal and mucous-platelet cells form part of the gill-water barrier, and are crossed by diffusing respiratory gases; Bayer intoxication does seem to cause these respiratory cells to swell slightly (Fig. 13), and this might interfere with branchial respiratory exchange. The rate of leakage of water or ions across basal and mucous-platelet cells could conceivably be accelerated when these cells are damaged by Bayer, disrupting ion-balance in the ammocoete.

It is intriguing that Bayer 73, when used with TFM as a synergist, both increases the effectiveness of TFM in killing ammocoetes and magnifies the extent of gill damage produced, especially the damage to ion-uptake cells. The TFM/Bayer mixture induces more than an additive increase in the rate of necrosis of these cells. Does this explain the synergistic action of Bayer? Should future synergists for TFM be sought among toxicants that preferentially affect gill structure?

In our laboratory, we have investigated the effects on lamprey gills of several toxic chemicals, including methyl mercury, acid water, and an organochlorine insecticide called Kepone. The lampricides, TFM and Bayer, are the only compounds we found to be highly selective for the ion-uptake cell type, and they cause more overall damage to ammocoete gills than do the other toxicants. All this supports the view that specific and severe alterations to ion-uptake cells contribute to the toxicity of TFM and Bayer to ammocoetes.

Mode of Selectivity of TFM and Bayer 73

Trout are more resistant to TFM and Bayer toxicity than are ammocoetes, and trout gills show no abnormalities at TFM/Bayer concentrations that kill ammocoetes and damage ammocoete gills. In fact, trout gills structure remains normal even at a TFM level lethal to the trout (30 mg/l), a concentration that is 600% of the lamprey 9 h LC 100. The

simplest interpretation of these findings is that trout are resistant to the lethal effects of lampricides because their gills show no effect.

This idea is may be too simplistic, because it fails to consider other evidence for the special susceptibility of ammocoetes to TFM (Lech and Statham 1975). Trout and other vertebrates, unlike lampreys, neutralize TFM by conjugating it with glucuronic acid in the liver; the conjugate is subsequently excreted from the animal in the bile and the urine. Bayer is similarly neutralized and excreted by teleosts (Howell et al. 1980). The findings of our present study can be used to expand this theory: We predict that lampreys, not able to neutralize and excrete TFM or Bayer via the biliary or renal route, must excrete the molecules in raw, toxic form across the gill via the ion-uptake cells. The subsequent gill poisoning and damage contributes to the death of the lamprey. Trout, on the other hand, would be excreting the harmless, neutralized conjugates via intestine and kidney (and via the gill?), so the trout gill structure would show no effect.

The main conclusion of this study is that ammocoete gills are markedly damaged by both TFM and Bayer, but trout gills are not.

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FIGURE LEGENDS

Figure 1. Gill lamella of sea lamprey ammocoete (unpoisoned control). Ion-uptake cells (IU), with dark mitochondria (MI), cover over 75% of the surface. Mucous-platelet cells (MP) cover the rest. B, basal cell; BL, blood sinus; LY, large secondary lysosome in a basal cell; P, pillar cell. x3,500.

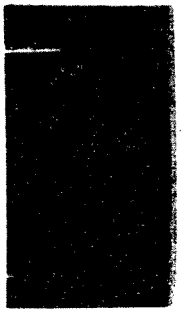
Figure 2. Ion-uptake cell (IU) in epithelium of normal ammocoete gill lamella. This cell has mitochondria (MI) with dense matrices and thin, parallel, cristae (arrowhead). Basal (B) and pillar (P) cells are also evident. G, Golgi apparatus; MV, microvilli. x22,400.

Figure 3. Tip of a normal ammocoete gill lamella, showing a mucous-platelet cell (MP) above, a basal cell (B) at lower right, and a white blood cell (L, lymphocyte) in the epithelium below. BL, blood sinus; G, Golgi apparatus; MI, mitochondrion, P, pillar cell; RER, rough endoplasmic reticulum; SG, mucous secretory granule. x22,400.

Figure 4. Ammocoete gill lamella affected by 2.5 mg/l TFM. The lamella is wider than that in Figure 1. Ion-uptake cells (IU) are hypertrophied (taller) and they contain vacuoles (V). Intercellular spaces (SP) are widened within the epithelium. x3,500.

ure 5. Ion-uptake cell (IU) from ammocoete affected by
mg/l TFM. Mitochondrial profiles (MI) are larger than
Figure 2, and many cristae have lost their parallel
angement (arrowheads). Microvilli are absent from cell
face (upper right), and a vacuole (V) is in the
oplasm. Underlying basal cell (B) is normal. x22,400.

ure 6. Tip of gill lamella from ammocoete exposed to 5.0
l TFM, in an alkaline water where effects are moderated.
n-uptake cell (IU) contains a vacuole (V), but
chondria (MI) appear normal. Basal (B) and mucous-
let cells (MP) are normal also. x7,000.



ure 7. Gill lamella from ammocoete exposed to 1.6 mg/l
M. Only a few small vacuoles (V) are evident in some ion-
take cells. x3,500.

ure 8. Gill lamella from ammocoete exposed to 55 ug/l
yer 73. Surface is lined more by mucous-platelet cells
P) than by ion-uptake cells (IU). Near the tip, one ion-
take cell is vacuolated (V), and the basal (B) and mucous-
atelet cells have lighter nuclei and lighter cytoplasms
an in control gills (cf. Fig. 1). x3,500.

gure 9. Ion-uptake cell from ammocoete exposed to 55 ug/l
yer 73. Note the big vacuole (V), the absence of
crovilli, and the Golgi apparatus (G) in whorls. At

right, some mitochondrial profiles (MI) seem enlarged. B, basal cell. x22,400.

Figure 10. Base of a lamella from ammocoete exposed to 55 ug/l Bayer 73. Two ion-uptake cells (IU) are necrotic; the one on the left is peeling off. V, vacuole. x3,500.

Figure 11. Mucous-platelet cell (MP) from tip of lamella of an ammocoete exposed to 55 ug/l Bayer 73. Nucleus is larger and whiter than in control cells of Figure 3, and cytosol appears lightened. x22,400.

Figure 12. Mucous-platelet cells affected by 55 ug/l Bayer 73, more damaged than in Figure 11. Cytosol is very light and Golgi apparatus (G) is scattered. Mitochondria (MI) are light, swollen, and have disoriented cristae. P, pillar cell; RER, swollen rough endoplasmic reticulum; SG, secretory granules; V, vacuole. x22,400.

Figure 13. Heavily affected gill lamella from ammocoete exposed to 55 ug/l Bayer 73. Very few ion-uptake cells (IU) are present. Most basal (B) and mucous-platelet (MP) epithelial cells contain a lightened cytosol and appear hypertrophied (cf. Fig. 1, control lamella). Epithelial nuclei are enlarged and appear light. The red blood cells (R) outside of the lamella were released upon sacrifice of

the animal and have nothing to do with Bayer 73 poisoning. x3,500.

Figure 14. Gill lamella from ammocoete exposed to a lethal TFM/Bayer mixture. a. top half of lamella; b. lamellar base. Right side of lamella is mostly lined by ion-uptake cells (IU), left side by mucous-platelet cells (MP). Three necrotic ion-uptake cells (IU_n) have left the lamella. x3,500.

Figure 15. Various epithelial cell types from ammocoete gill lamella affected by a lethal TFM/Bayer mixture. Basal cells (B) appear normal here. The ion-uptake cell (IU) has swollen, distorted mitochondria (MI), a large vacuole (V), and a vacuolated Golgi apparatus (G). At left floats a dead ion-uptake cell (IU_n). x22,400.

Figure 16. Mucous-platelet cell from ammocoete gill lamella affected by TFM/Bayer. This cell contains clusters of vacuoles (V). At upper right, a vacuole occurs in a junctional complex (J). P, pillar cell. x22,400.

Figure 17. Tip of ammocoete gill lamella affected by TFM/Bayer. Mucous-platelet (MP) and basal (B) cell are shown. Nuclei appear enlarged and light. Cytoplasm is lighter than in controls (cf. Figure 3). V, vacuole. x22,400.

Figure 18. Trout gill lamella, control. This particular lamella is thicker than most gill lamellae in section, due to presence of many chloride cells (C); however, it is still within the range of normal appearance. B, basal cell; BL, blood cell in blood sinus; Pa, pavement cell; P, pillar cell. x3,910.

Figure 19. Normal trout gill, enlargement of the lamellar tip in Figure 18. Chloride cell (C) has large mitochondria (MI) and cytoplasmic tubules (T). Pavement cell (Pa) exhibits cisterns of endoplasmic reticulum (ER) plus clear vesicles (CV). P, pillar cell; R, red blood cell. x12,400.

Figure 20. Lamella of trout exposed to 30 mg/l TFM. Gill appears normal. Abbreviations as in Figure 18. x3,910.

Figure 21. Trout pavement cell from lamella of Fig. 20, exposed to 30 mg/l TFM. Appearance is normal. Abbreviations as in Figure 19. x26,600.

Figure 22. Trout chloride cell from lamella of Figure 20, exposed to 30 mg/l TFM. Appearance is normal. Abbreviations as in Figure 19. x26,600.

Figure 23. Three gill lamellae from trout exposed to 55 ug/l Bayer 73. No abnormalities are evident. Abbreviations as in Figure 18. x3,910.

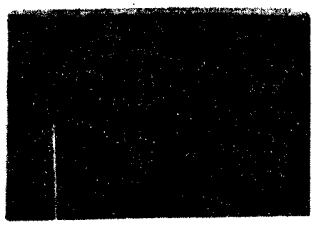


TABLE 1. Exposure Conditions Used in this Study, and Number of Animals Examined per Exposure.

Concentration of Lampricide	TFM	Bayer 73	TFM/Bayer Mixture
I. The ammocoete 9 h LC100			
	A. Ammocoetes 1. Michigan (2.5 mg TFM/l) a. three well larvae (exposed 4 - 5.5 h) b. three sick larvae (exposed 2.5 - 6 h) c. two control larvae (in clean water 9 h) 2. Washington (5 mg TFM/l) a. two well larvae (exposed 9 h) b. one sick larva (exposed 6 h) c. one control larva (in clean water 9 h)	A. Ammocoetes 1. Michigan (55 ug Bayer/l) a. five well larvae (exposed 5.5 - 7 h) b. five sick larvae (exposed 5 - 6 h) c. three control larvae (in clean water 9 h)	A. Ammocoetes 1. Michigan (1.25 mg TFM, 20 ug Bayer/l) a. four well larvae (exposed 7 h) b. five sick larvae (exposed 4 - 7.5 h) c. four control larvae (in clean water 9 h) 2. Washington (1.9 mg TFM, 23.75 ug Bayer/l) a. one sick larva (exposed 7 h)
	B. Trout 1. Washington (5 mg TFM/l) a. three well trout	B. Trout* 1. Michigan (55 ug Bayer/l) a. one well trout	B. Trout 1. Washington (1.9 mg TFM,

II. The trout 9 h LC100

TFM	Bayer 73	TFM/Bayer Mixture
A. Trout 1. Washington (30 mg TFM/1) a. three well trout (exposed 9 h) b. one sick trout (exposed 2 h) c. one control trout (in clean water 9 h)	-----	-----

III. The lamprey 96 h LC50

TFM	Bayer 73**	TFM/Bayer Mixture
A. Ammocoetes 1. Michigan (1.6 mg TFM/1) a. three well larvae (exposed 12 h) b. two control larvae (in clean water 12 h)	A. Ammocoetes 1. Michigan (36 ug Bayer/1) a. five well larvae (exposed 12 h) b. four control larvae (in clean water 12 h)	-----

*In Michigan trout, fixation of gills was sub-optimal

**Light microscopic sections only