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COMPARATIVE STUDY OF THE EFFECTS OF MCPA, BUTYLATE, ATRAZINE, AND CYANAZINE ON *SELENASTRUM CAPRICORNUTUM*

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Abstract

The herbicides MCPA, butylate, atrazine and cyanazine are extensively used in Canadian agriculture and information regarding their effects on indigenous biota is scarce. Phytotoxicity assessments were conducted in the laboratory on the common green alga Selenastrum capricornutum *using both the active ingredient of the herbicides and their formulated products (for MCPA and butylate). Endpoints determined after the 96 h exposure included algal population growth inhibition (IC_{50}—cell counts), percent lethality (LC_{50}—flow cytometry derived) and photosynthetic electron transport inhibition (EC_{50}—fluorescence induction).*

Pesticide formulations had greater toxic effects than the active ingredient alone. The 96 h IC_{50} (50% Inhibition Concentration) and LOEC (Lowest Observable Effects Concentration) using cell counts of S. capricornutum *were 18.4 and 8.9 mg l^{-1} respectively for MCPA-active ingredient and for MCPA-formulated, these were 0.62 and 0.0062 mg l^{-1} respectively. Those for butylate-active ingredient were 61.0 and 8.3 mg l^{-1} and for butylate-formulated 1.46 and 0.17 mg l^{-1}, respectively. The triazines active ingredient, which are photosynthetic inhibitors, had greater effects than either the MCPA or butylate. The IC_{50} for cyanazine and atrazine were 0.059 and 0.026 mg l^{-1}, respectively.*

By comparing the IC_{50} and LC_{50} values for the tested active ingredients, it was found that the effects of atrazine were algicidal, whereas those of cyanazine, butylate and MCPA were algistatic. Copyright © 1996 *Elsevier Science Ltd.*

INTRODUCTION

Herbicide phytotoxicity assessments

Several reports, documents and databases summarize pesticide usage in Canada's agricultural regions (Environment Canada/Agriculture Canada, 1987; Cossette *et al.*, 1988; Environment Canada, 1993). These base their findings on sales data, known fate and toxicity profiles and proximity of usage to water bodies. Of high priority, in terms of their potential impacts on the aquatic environment, are the thiocarbamate herbicide butylate, the phenoxy acid herbicide MCPA and the triazines, atrazine and cyanazine. Butylate is used primarily against annual grasses in field and sweet corn, whereas MCPA is effective at controlling broadleaf weeds in cereals, forage crops, peas and flax. The triazines are used to control both broadleaf and grassy weeds on agricultural crops including corn, sorghum, sugarcane and pineapple. Since 1982, the use of these herbicides has increased greatly in Canada. For example, MCPA is currently among the top four most heavily used pesticides in Canada, with its principal usage occurring in the prairie regions (Lewis, 1989). Nationwide, MCPA has been measured in three southern Ontario watersheds and rivers in Saskatchewan (max. 13.2 $\mu g\ l^{-1}$) and Alberta (Frank & Logan, 1988; National Water Quality Database NAQUADAT, 1993). The freshwater aquatic life Canadian water quality guideline for MCPA is 2.6 $\mu g\ l^{-1}$. The triazines are found annually in many Canadian fresh water systems (e.g. 40 $\mu g\ l^{-1}$) (Forrest & Caux, 1990) at concentrations that greatly exceed the Canadian Water Quality Guidelines of 2 $\mu g\ l^{-1}$ (Trotter *et al.*, 1990). Considering the high uses of these herbicides within large agricultural regions in Canada, there is a surprising dearth of information regarding their effects on indigenous biota, in particular non-target plants (Constable, 1988; Kent *et al.*, 1991). This lack of basic information has also contributed to the initiation of the regulatory re-evaluation of MCPA and atrazine in Canada.

Non-target plant toxicological assessment has been recently identified by the Canadian Department of the Environment as a priority area in pesticide registration and re-evaluation (Swanson & Peterson, 1988). Most phytotoxicological research with herbicides has been conducted on target plants (i.e. efficacy studies on weeds). Little data exist on the effects these pollutants may have in aquatic systems. There is an essential need

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to assess the basic phytotoxicity of these herbicides on non-target aquatic plants which form the basis of energy flow in aquatic food webs. This report summarizes an investigation evaluating the phytotoxic potential of these high use compounds on common phytobiota models. Phytotoxicity assessments were conducted in the laboratory on the common green algae *S. capricornutum* using butylate and MCPA (technical and end-use products) and the triazines, atrazine and cyanazine.

Algal bioassays

Unicellular algae have the advantage of being sensitive to xenobiotics. They have a relatively short life cycle with exponential growth curves and are easily manipulated in the laboratory. Chlorophycea, diatoms and several cyanophytes are unicellular algae that have these toxicological and analytical characteristics (Jouany, 1981; Blaise, 1991). In 1978, the United States Environmental Protection Agency published an algal phytotoxicity bioassay protocol using *S. capricornutum* as the algae of choice (Miller *et al.*, 1978). This protocol was miniaturized from the use of Erlenmeyer flasks to the use of microplates rendering it faster, easier to handle, more economical, and due to the larger number of replicates, a statistically more robust test. Investigations using *S. capricornutum* and microplates have been used in the determination of pesticide (Blanck *et al.*, 1984; Blaise & Harwood, 1991; St-Laurent *et al.*, 1991; Caux *et al.*, 1992), heavy metal (Hassett *et al.*, 1981; Couture *et al.*, 1989; Thellen *et al.*, 1989) and industrial effluent (Joubert, 1980; Couture, 1981; Blaise *et al.*, 1986; Environment Canada, 1992) toxicity.

The current experiments were performed in microplates using *S. capricornutum* and consisted of measuring algal growth inhibition with an electronic particle counter, algal photosynthetic effects using a fluorometer and algal lethality using a flow cytometer.

Flow cytometry

Flow cytometry is a measure of optical signals emanating from individual cells in a liquid flow. Once a biomedical research tool, it has since been adapted to aquatic ecosystem research (Yentsch *et al.*, 1984; Legendre & Yentsch, 1989; Phinney & Cucci, 1989; Premazzi *et al.*, 1989).

Algal cellular viability is obtained by a measure of the enzymatic esterase activity (marker induced) and chlorophyll fluorescence. By plotting these measures against each other and comparing the values to those of controls, viable and non-viable populations of cells fall into distinct 'windows' and are easily distinguishable. This technique is advantageous over particle counting which does not discriminate between live and dead cells. Thus, with reference to both algal growth potential and toxicity assays, a response parameter should reflect both non-lethal and lethal stress (Rehnberg *et al.*, 1982). For a more in-depth review of the subject area, the reader is referred to appropriate review articles (Forrest, 1984; Steinkamp, 1984; Muirhead *et al.*, 1985).

Fluorometry

The fluorometric principle relies on the excitation of the chlorophyll molecules at a wavelength concurrent with their absorption maxima. The emitted fluorescent light is then filtered, amplified and recorded. The toxicological significance for a bioassay using chlorophyll fluorescence as an end-point is in the modification of the normal fluorescence induction curve. The induction curve can be modified by any factors affecting one or more transients of the curve. For example, S-triazine herbicides associate with a 32 kDa protein in complex B of photosystem II which has the effect of blocking and inhibiting the transfer of electrons between quinone acceptors. This inhibition results in a strong increase of the fluorescence emission (Kautsky effect) and can be detected efficiently by the utilization of a fluorometer (Caux *et al.*, 1992). The use of fluorescence generated EC_{50} for chemicals inhibiting the biological energy transfer mechanism in photosynthesis is regarded to be a more sensitive toxic parameter than growth generated EC_{50} (Blanck *et al.*, 1984; Caux *et al.*, 1988).

Formulations

Experimental formulations are often undergoing changes in order to enhance the efficacy and specificity of the active ingredient (Caux & Weinberger, 1993). Currently, in Canada, much of the data requirements in support of product registration deal with the active ingredient and not the formulation (Plant Industry Directorate, 1993). Often, formulation experts and manufacturers consider adjuvants as inert ingredients in the formulations. Studies have shown that adjuvant use has a potential to generate toxicity to a wide range of organisms (Caux *et al.*, 1986) at all levels of cellular organization (Caux *et al.*, 1988, 1993; Premazzi *et al.*, 1989). When conducting toxicological studies on pesticides, it is important to report responses due to the active ingredient and the formulated product and to discern between effects attributed to adjuvants and/or the active ingredient.

MATERIALS AND METHODS

Pesticide products, test protocols and test organisms

Amine 500 (MCPA EC, 50% active ingredient) was obtained from Ciba-Geigy, Mississauga, Ontario, Canada. MCPA acid (96% active ingredient) was obtained from Dowelanco, Newmarket, Ontario and a second stock from Reidel de Haën, Germany (98%). Butylate (96.8% active ingredient) was obtained from ICI Chipman, Stoney Creek, Ontario and a second stock from Reidel de Haën (98%). Sutan (butylate EC, 80% active ingredient) was obtained from Stauffer Inc., Montreal, Quebec, Canada. Atrazine and cyanazine were obtained from Reidel de Haën.

Other reagents, growth media, test protocols and instrumentation, have been described elsewhere (Miller *et al.*, 1978; Blaise *et al.*, 1982; Métézeau & Frelat, 1991; Legendre & Yentsch, 1989; Thellen *et al.*, 1989;

Ménard, 1991). The following is a brief description of these experimental procedures.

Test organisms

The test organism used was the unicellular chlorophycea *S. capricornutum*. These were obtained from the E.P.A. Corvallis Environmental Research Laboratory, Corvallis, Oregon. Axenic stock cultures were kept in 4 l Erlenmeyer flasks in AAM (Algal Assay Medium (Swanson and Peterson, 1988)). Cultures were grown in incubators with continuous light condition of 60 $\mu E/m^2/s$, at a temperature of 24 ± 1°C and shaking at 100 rpm.

Inoculation and treatment procedures for *S. capricornutum*

The inoculation and treatment procedures used follow those previously described in Environment Canada's biological Test Method's report (Environment Canada, 1992). From a stock culture at its logarithmic growth phase (4–7 days), 10–15 ml were centrifuged (1000 g, 10 min) to clean up the cellular debris. The pellet was resuspended in 5 ml of $NaHCO_3$ (15 mg in 1 l of deionized distilled water). A 10 µl aliquot of resuspended cells was placed in a 20 ml cuvette containing 10 ml of HEMATALL isotonic solution. The cells were counted with the electronic cell particle counter (Coulter model ZM) and the dilution factor calculated to obtain a concentration of 220 000 cells/ml (stock 1). In each well of the microplate, 10 µl of stock 1, 10 µl AAM nutrient solution and 200 µl deionized distilled water (220 µl total) gave a 10 000 cells/ml initial concentration for the 4 day tests enabling an optimum count (1–2 x 10^6 cells/ml) following the incubation period in control sets. Following preliminary concentration range-finding tests, cells were exposed to the four pesticide products (the active ingredient for butylate and MCPA and their respective formulated products) in separate investigations. The microplate experimental layout of algal inoculum and treatment follow that of St-Laurent *et al.*, 1991 and is shown in Fig. 1.

Particle counts (IC_{50})

The method for electronic cell enumeration followed that previously described in Environment Canada's biological Test Method's report (Environment Canada, 1992). The aperture diameter was 70 µm. Cells were resuspended and 170 µl were dispensed into individual plastic cups filled with 10 ml isotonic diluent. Samples containing algae were counted at least three times with the particle counter.

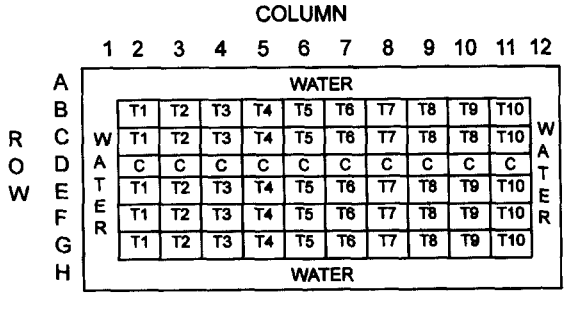

Fig. 1. Microplate configuration. Peripheral wells contain 200 µl of deionized water. T1 and T10 are the highest and lowest toxin (pesticide) concentrations, respectively. Row D is a control inoculum.

Flow cytometry (LC_{50})

A Becton Dickinson Flow Cytometer (model FACScan) was used to determine the 96 h LC_{50} of the pesticides on *S. capricornutum*. Cellular populations were analyzed using the fluorescent probe fluorescein diacetate (FDA) (530 nm) and the natural fluorescence emitted by the chlorophyll pigments (650 nm). Cytometric readings were taken before and after FDA addition. This procedure discriminated between an increase in chlorophyll fluorescence and FDA fluorescence as it is known that damaged photosynthetic systems may change their emission spectrum thereby creating false positives in the FDA emission spectra (Berglund & Eversman, 1988; Gala & Giesy, 1990).

Fluorescence (EC_{50})

An evaluation of indirect effects of butylate and MCPA on photosynthesis were conducted using fluorescence measurements with algae grown in microplates.

Fluorescence readings of algae in microplates were made on a Fluorometer model Fluoroskan II by Labsystems, Helsinki, Finland. The fluorometer was set to recalibrate at 0 after each reading (100 ms per well). The excitation and emission wavelengths were set at 430 and 660 nm, respectively. The fluorescence was measured as relative fluorescence units (R.F.U.) (Caux *et al.*, 1992).

Data analysis

All experiments were conducted at least twice at two different time periods. Homogeneity of variance was determined using Bartlett's test (Bartlett, 1937). Analyses of variance (ANOVA) were performed on the raw data of all treatments. A comparison of the means between treatments was included (Bonferroni multiple range test). Significance was obtained at $p < 0.05$. Estimation of the LC_{50} was done by linear regression, and a Bonferroni test ($p < 0.05$) was used to determine the LOEC. The esterase activity was estimated with the use of a Student t test.

RESULTS

Results of the eight plots (not shown) of the adjusted probits (EPA Probit Analysis Program, ver. 1.4) and calculated regression lines for *S. capricornutum* exposed (96 h) to varying concentrations of MCPA, butylate and their formulated product demonstrate significant differences in cell counts between these treatments. To summarize these, the IC_{50} (50% inhibition concentration) and LOEC (lowest observable effect concentration) have been calculated from the probit analysis and are illustrated in Fig. 2. The summary of the probits for the 96 h algal fluorescence results given as EC_{50} and

LOEC for the two above active ingredient and their formulation is illustrated in Fig. 3.

Flow cytometry results from a population of non-viable and viable algal cells are presented in Fig. 4. A plot of the green fluorescence following FDA staining versus the red chlorophyll fluorescence has discriminated between these two populations. To better identify the populations, the non-viable cells have been assigned to window No. 1 and the viable cells to window No. 2.

From these windows, a percentage of the non-viable and viable cells can be obtained for each of the treatment concentrations. The LC_{50} values were obtained by simple linear regression of the dose response curves (Fig. 5). In this illustration, the regression lines have not been drawn. The x axis representing the pesticide concentration has been logged to better visualize the four curves. A summary of the 96 h LC_{50} and 96 h IC_{50} results for MCPA, butylate, cyanazine and atrazine is presented in Table 1. Note that these latter investigations were conducted with Reidel de Haën products active ingredient which were a purer grade of MCPA and butylate. Observed is the fact that the IC_{50} toxicity values for MCPA and butylate active ingredient coming from two different manufacturers varied (Fig. 2).

DISCUSSION

Of the data available in the literature, the aquatic organisms which demonstrated greatest sensitivity to MCPA were the green algae *S. capricornutum* with a 5 day EC_{50} of 0.19 mg l^{-1} and a LOEC of 0.033 mg l^{-1} (CCREM, 1987). The literature reports green algal EC_{50} for MCPA up to 500 mg l^{-1} (Kirkwood & Fletcher, 1970). Other toxicological studies on non-target plant (terrestrial) species are available and report EC_{50} (48 h to several weeks) ranging from 0.2–166 mg l^{-1} (Eliasson, 1963; Lyndsay & Hartley, 1966; Zsoldos & Hanunold, 1978; Phytotox, 1989). From a review of the literature (CCREM, 1987) plants may be the most susceptible biota to this herbicide, however, this sensitivity varies considerably within a phytobiota class.

The 96 h IC_{50} of 18.4 mg l^{-1} and LOEC of 8.9 mg l^{-1} for MCPA observed in these investigations is within the range of those previously reported. The IC_{50} for the

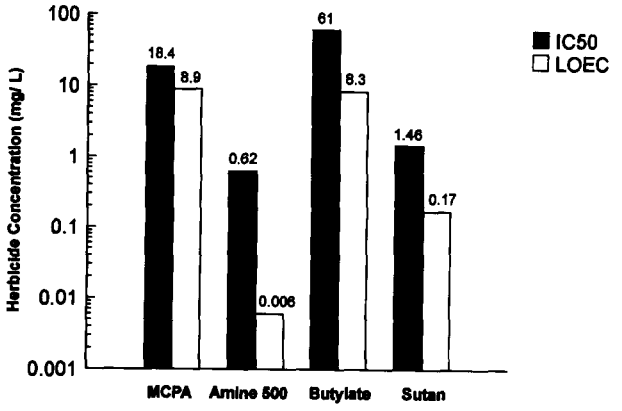

Fig. 2. IC_{50} and LOEC (cell counts) for MCPA, butylate and their formulated products in mg l^{-1}. Values calculated from the EPA Probit Analysis Program (Version 1.4). Pesticides obtained from Ciba-Geigy, Dowelanco, ICI Chipman and Stauffer (see text).

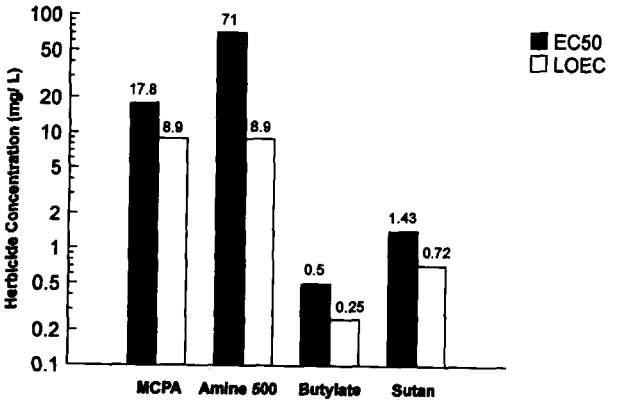

Fig. 3. EC_{50} and LOEC (fluorescence) for MCPA, butylate and their formulated products in mg l^{-1}. Pesticides obtained from Ciba-Geigy, Dowelanco, ICI Chipman and Stauffer (see text).

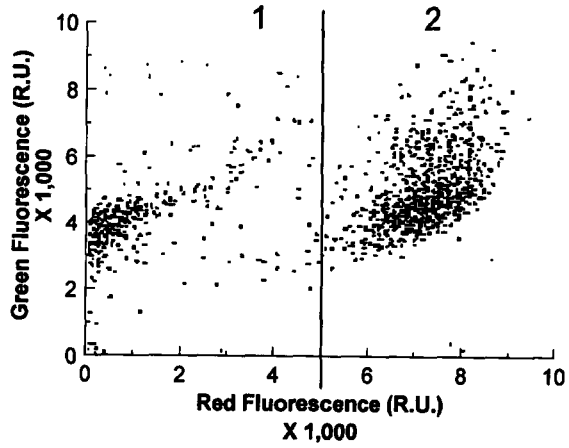

Fig. 4. Flow cytometry cytogram. Abscissa and ordinate values are relative units (R.U.) of green (530 nm) and algal chlorophyll red (620 nm) fluorescences, respectively, after incubation with FDA. The non-viable and viable cell populations are depicted in windows 1 and 2, respectively.

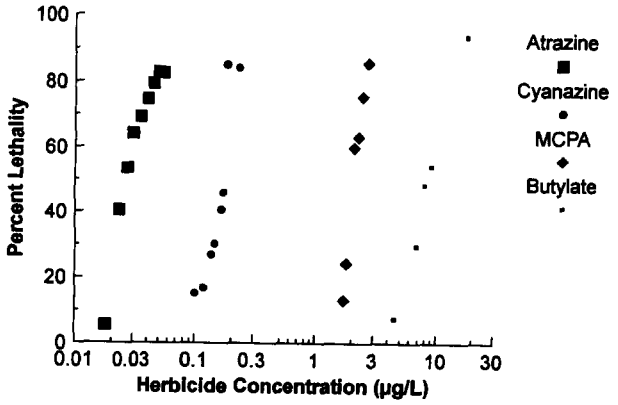

Fig. 5. Flow cytometry dose response curves (LC_{50}) for atrazine, cyanazine, MCPA and butylate. Pesticides obtained from Riedel de Haën.

Table 1. Pesticide 96 h LC_{50} (mg l^{-1}) and 96 h IC_{50} (mg l^{-1}) from simple linear regression of the dose response curves obtained from flow cytometry and particle counter, respectively

	Pesticide[d]			
	MCPA	Butylate	Cyanazine	Atrazine
LC_{50}	2.13	9.79	0.145	0.026
95% C.I.[a]	1.85–2.38	5.55–14.04	0.126–0.207	0.023–0.027
Y intercept	−106.16	−6.03	−77.89	6.77
Slope	73.3	5.73	0.82	1.69
r^2[b]	95%	93%	81%	92%
n[c]	4	4	3	3
IC_{50}	1.94	6.47	0.059	0.026
95% C.I.[a]	1.78–2.10	0.94–13.53	0.047–0.072	0.019–0.032
Y intercept	−101.55	25.01	8.18	−78.44
Slope	78.12	3.87	0.70	4.95
r^2[b]	98%	82%	97%	91%
n[c]	3	3	3	3

[a] Confidence Interval.
[b] Correlation Coefficient.
[c] Replicates.
[d] Obtained from Riedel de Haën.

formulated product of MCPA (Amine 500) was 0.62 mg l^{-1} and LOEC 0.006 mg l^{-1}. This latter value is three orders of magnitude below the pure compound's LOEC indicating that toxicological testing should be performed with the formulation leading to a more realistic account of herbicide exposure. Similarly *S. capricornutum* demonstrated more than one order magnitude greater sensitivity to the formulated butylate. The 96 h IC_{50} for butylate was 61 mg l^{-1} and for its formulated product 1.46 mg l^{-1}.

The water solubility of MCPA and butylate is 825 and 46 mg l^{-1}, respectively. The guaranteed content in the formulated product is 500 and 800 g l^{-1}, respectively. To obtain these concentrations emulsifiers and/or solvent carriers must be used. Adjuvant or formulation additives have been the subject of numerous toxicity investigations (Hodgson & Maryland, 1982; Caux & Weinberger, 1993; Plant Industry Directorate, 1993). It has been shown that some of these alone (i.e. without the active ingredients and/or other adjuvants) are phytotoxic at field relevant concentrations (Weinberger & Greenhalgh, 1984). Subtle changes in the adjuvants physicochemical characteristics can alter their phytotoxicity (Plant Industry Directorate, 1993). In a formulation, adjuvants not only act as solubilizers and emulsifiers but often enable the active ingredient to get to the target site of action. It has been shown that, at field relevant concentrations, adjuvants can increase plant membrane fluidity thereby facilitating the penetration of the active ingredient (Caux & Weinberger, 1993; Plant Industry Directorate, 1993). To date, phytotoxicity studies required for pesticide registration are still conducted on the active ingredient with an appropriate solvent (acetone). As adjuvants play a predominant role in determining the environmental chemistry, fate and toxicology of the active ingredient, more emphasis should be placed on regulatory phytotoxic investigations focused on the formulations. In the current investigation, tests on formulations alone were not conducted as these are guarded secrets of the manufacturers.

The formulated product of MCPA (Amine 500), using fluorescence as an end-point has resulted in an EC_{50} (71 mg l^{-1}) and a LOEC (8.9 mg l^{-1}) that are one and three orders of magnitude above those using cell counts as an end-point, respectively. Analytical MCPA results are comparable with both end-points. This suggests that electron transport inhibition may not be as sensitive of a parameter as cell counts when monitoring for toxicological effects and is exemplary of the fact that MCPA acts as an anti-auxin interfering with normal plant growth.

Butylate on the other hand, a compound known to interfere with lipid biosynthesis, displayed a greater inhibitory effect when using fluorescence as an end-point as compared to cell counts. The normal functioning of photosystems and their associated transport chains are intimately linked with lipid domain integrity. It is speculated that butylate by inhibiting the formation of long-chain fatty acids in isolated chloroplasts (Wilkinson & Smith, 1975) would disrupt normal photosynthetic functioning thereby increasing the fluorescence yields.

Tests with the triazine herbicides atrazine and cyanazine demonstrated that these compounds are one to two orders of magnitude more phytotoxic than either MCPA or butylate. The triazines impart their toxicities by complexing with the 32 kDa protein in complex B of photosystem II in chloroplast photosynthetic membranes (Schulz *et al.*, 1990). The result is a block in the electronic transport chain translating to significant increases in the fluorescence (Caux *et al.*, 1992). This lends itself well to phytotoxicological investigation by flow cytometry. The authors are not aware of other phytotoxicological studies reporting triazine effects by flow cytometry. The reported LC_{50} for atrazine (0.026

mg l^{-1}) and cyanazine (0.145 mg l^{-1}) herein, compare to or are lower than those of the most sensitive values reported elsewhere for green algae (Trotter *et al.*, 1990; Pauli *et al.*, 1991).

By comparing the LC_{50} and IC_{50} values (Table 1) for MCPA, butylate, cyanazine and atrazine, it can be determined that only atrazine has algicidal type toxicity to *S. capricornutum*. The other herbicides have an algistatic type toxicity. In the latter toxicity type, toxicity tests are sublethal and are designed to give information on cellular stress. In contrast, when cells have been stressed beyond their resilience capacity disabling their recovery or acclamation to the stressor giving rise to cellular death, the stressor is referred to as algicidal. Toxicity tests of this type are lethal tests (Ménard, 1991).

In summary, in these investigations, bioassay endpoints were (1) algal lethality (LC_{50}) measured by flow cytometry, (2) algal growth inhibition (IC_{50}) measured by cell counts and (3) algal photosynthetic effects (EC_{50}) measured by fluorometry. These different parameters enabled differentiation between sublethal and lethal effects for MCPA, butylate, atrazine and cyanazine, four herbicides heavily used in Canada. Tests with MCPA and butylate technical active ingredient versus their formulations clearly showed that the formulations were orders of magnitude more phytotoxic than the active ingredient

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REFERENCES

Bartlett, M. S. (1937). Some examples of statistical methods research in agriculture and applied biology. *J. Roy. Statist. Soc. Suppl.*, **4**, 137–70.

Berglund, D. L. & Eversman, S. (1988). Flow cytometric measurement of pollutant stresses on algal cells. *Cytometry*, **9**, 150–5.

Blaise, C. (1991). Microbiotests in aquatic ecotoxicology: characteristics, utility, and prospects. *Environ. Toxicol. Water Qual.*, **6**, 145–55.

Blaise, C. & Harwood, M. (1991). Contribution à l'évaluation écotoxicologique du Tébuthiuron—un herbicide de la classe des urées substitués. *Rev. Sci. Eau*, **4**, 121–34.

Blaise, C., Legault, R. & Bermingham, N. (1982). A simple microassay technique for measuring algal inhibition (EC_{50}) in aquatic toxicity studies *Can. Tech. Rep. Fish. Aquat. Sci.*, **1163**, 1–8.

Blaise, C., Legault, R., Bermingham, K., Van Coillie, R. & Vasseur, P. (1986). A simple microplate algal assay technique for aquatic toxicity assessment. *Toxic. Assess.*, **1**, 261–81.

Blanck, H., Wallin, G. & Wängberg, S-Å. (1984). Species-dependent variation in algal sensitivity to chemical compounds. *Ecotoxicol. Environ. Safe.*, **8**, 339–51.

CCREM (1987). *Canadian Water Quality Guidelines*. Prepared by the Task Force on Water Quality Guidelines of the Canadian Council of Resource and Environment Ministers. Ottawa, Ontario.

Caux, P.-Y., Blaise, C., Leblanc, P. & Taché, M. (1992). A new phytoassay procedure using fluorescence induction. *Environ. Toxicol. Chem.*, **11**, 549–57.

Caux, P.-Y. & Weinberger, P. (1993). A comparative physiological study of the effects of four Triton surfactants on Lemna minor. Effects on lipid classes. *Environ. Pollut.*, **81**, 151–6.

Caux, P.-Y., Weinberger, P. & Carlisle, D. B. (1986). Dowanol, an environmentally safe adjuvant. *Environ. Toxicol. Chem.*, **5**, 1047–54.

Caux, P.-Y., Weinberger, P. & Carlisle, D. B. (1988). A physiological study of the effects of Triton surfactants on Lemna minor L. *Environ. Toxicol. Chem.*, **7**, 671–6.

Caux, P.-Y., Weinberger, P. & Szabo, A. (1993). A comparative physiological study of the effects of four Triton surfactants on Lemna minor. Effects on lipid dynamics. *Can. J. Bot.*, **17**, 1291–7.

Constable, M. (1988). *Summary of the Review of Environmental Information Pertaining to the Use of MCPA in the Western and Northern Region*. Scientific Programs Branch Report, Environmental Protection, Edmonton, Alberta, Canada.

Cossette, D., Giroux, I. & Poulin, R. (1988). *Recueil des Principaux Pesticides en Usage au Québec*, SAGE Ltd., Report prepared for Environment Canada, Inland Waters Directorate, Water Quality Branch, Montreal, Quebec, Canada.

Couture, P. (1981). PhD Thesis, Paul Sabatier University, Toulouse, 128 pp.

Couture, P., Blaise, C., Cluis, D. & Bastien, C. (1989). Zirconium toxicity assessment using bacteria, algae, and fish assays. *Water Air Soil Pollut.*, **47**, 87–100.

Eliasson, L. (1963). The toxic effects of chlorinated phenoxyacetic acid on aspen. *Physiol. Plant.*, **16**, 255.

Environment Canada. (1992). Report EPS 1/RM/25.

Environment Canada. (1993). *A Catalogue of Ontario Agro-Ecosystem Databases*. Environment Canada, Ottawa, Ontario, Canada.

Environment Canada/Agriculture Canada. (1987). *Pesticide Registrant Survey*. 1986 Report. Commercial Chemicals Branch, Conservation and Protection, Environment Canada, Ottawa, Ontario, Canada.

Forrest, J. I. M. (1984). Possible applications of flow cytometry in the aquatic sciences. *Eur. Appl. Res. Rep. Environ. Nat. Resour. Sect.*, **2**, 387–400.

Forrest, S. & Caux, P.-Y. (1990). *Pesticides in Tributaries of the St. Lawrence River 1987–1988 Program Report*, St. Lawrence Centre, Montreal, Quebec, Canada.

Frank, R. & Logan, L. (1988). Pesticide and industrial chemical residues at the mouth of the Grand, Saugeen, and Thames Rivers, Ontario, Canada, 1981–1985. *Arch. Environ. Contam. Toxicol.*, **17**, 741–54.

Gala, W. & Giesy, J. P. (1990). In *Aquatic Toxicology and Risk Assessment*, Vol. 13, ed. L. van der Schalie. ASTM, Baltimore, pp. 237–46.

Hassett, J. M., Jennett, I. C. & Smith, I. E. (1981). Microplate technique for determining accumulation of metals by algae. *Appl. Environ. Microbiol.*, **41**, 1097–106.

Hodgson, R. H. & Maryland, F. (1982). *Adjuvants for Herbicides*. Weed Science Society of America, Champaign, IL.

Jouany, J. M. (1981). Les tests d'écotoxicité aiguë chez les algues et les macrophytes, In *Compte Rendu du Colloque International d'Écotoxicologie*, ed. H. Leclerc and D. Dive. Inst. Nat. Santé Rech. Méd., France, pp. 169–81.

Joubert, G. (1980). A bioassay application for quantitative toxicity measurements, using the green alga *Selenastrum capricornutum*. *Water Res.*, **14**, 1759–63.

Kent, R. A., Moore, D. R. J. & Walker, S. L. (1991). Data gaps in the aquatic toxicology of priority pesticides and industrial toxic substances in Canada. *Can. Tech. Rep. Fish. Aquat. Sci.*, **1774**, 1111–24.

Kirkwood, R. C. & Fletcher, W. W. (1970). Factors influencing the herbicidal efficiency of MCPA and MCPB in three species of micro-algae. *Weed Res.*, **10**, 3–10.

Legendre, L., & Yentsch, C. M. (1989). Overview of flow cytometry and image analysis in biological oceanography and limnology. *Cytometry*, **10**, 501–10.

Lewis, T. (1989). *Herbicide Use Survey*, Manitoba Department of Agriculture: Economics Branch, 908-401 York Avenue, Winnipeg, Manitoba, Canada.

Lyndsay, R. V. & Hartley, G. S. (1966). Studies on the response of plants to root-applied herbicides: further observations on the effect of localized application. *Weed Res.*, **6**, 221–32.

Ménard, L. (1991). Masters Thesis, Université du Québec, Institut national de la recherche scientifique, Sainte-Foy, Québec, Canada, 191 pp.

Métézeau, P. & Frelat, G. (1991). Principes, potentialés et limites de la cytométrie en flux. Applications en microbiologie. *Ann. Biol.* pp. 39–57.

Miller, W. E., Greene, J. C. & Shiroyama, T. (1978). The *Selenastrum capricornutum* Printz algal assay bottle test: Experimental design, application, and data interpretation protocol. In *United States Environmental Protection Agency Report No. EPA-60019-78018*, Corvallis, Oregon, 126 pp.

Muirhead, K. A., Horan, P. K. & Poste, G. (1985). Flow cytometry: present and future. *Biotechnology*, **3**, 337–55.

National Water Quality Database (NAQUADAT), (1993). Environment Canada, Ottawa, Ontario, Canada.

Pauli, B. D., Kent, R. A. & Wong, M. P. (1991). *Canadian Water Quality Guidelines for Cyanazine*, Scientific Series No. 180, Inland Waters Directorate, Water Quality Branch, Ottawa, Ontario.

Phinney, D. A. & Cucci, T. L. (1989). Flow cytometry and phytoplankton. *Cytometry*, **10**, 511–21.

Phytotox. (1989). Chemical Information System Database, Baltimore, MD. U.S.A.

Plant Industry Directorate. (1993). *Regulatory Directive Dir93-03*, Agriculture and Agrifood Canada, Ottawa, Ontario, Canada, 19 pp.

Premazzi, G., Buanonaccorsi, G. & Zillio, P. (1989). Flow cytometry for algal studies. *Water Res.*, **23**, 431–42.

Rehnberg, B. G., Schultz, D. A. & Raschke, R. L. (1982). Limitations of electronic particle counting in reference to algal assays. *Wastewater Anal.*, **54**, 181–6.

Schulz, A., Wengenmayer, F. & Goodman, H. M. (1990). Genetic engineering of herbicide resistance in higher plants. *Plant Sci.*, **9**, 1–15.

St-Laurent, D., Blaise, C., MacQuarrie, P., Scroggins, R. & Trottier, B. (1991). Comparative assessment of herbicide phytotoxicity to *Selenastrum capricornutum* using microplate and flask bioassay procedures. *Environ. Toxicol. Water Qual.*, **7**, 1–14.

Steinkamp, J. A. (1984). Flow cytometry. *Rev. Sci. Instrum.*, **55**, 1375–400.

Swanson, S. & Peterson, H. (1988). *Development of Guidelines for Testing Pesticide Toxicity to Non-target Plants*. SRC Publication E-901-20-E-88, Environment Canada, Ottawa, Ontario, Canada.

Thellen, C., Blaise, C., Roy, Y. & Hickey, C. (1989). Round robin testing with the *Selenastrum capricornutum* microplate toxicity assay. *Hydrobiologia*, **188/189**, 259–68.

Trotter, D. M., Baril, A., Wong, M. P. & Kent, R. A. (1990). *Canadian Water Quality Guidelines for Atrazine*, Scientific Series No. 168, Inland Waters Directorate, Water Quality Branch, Ottawa, Ontario.

Weinberger, P. & Greenhalgh, R. (1984). In *Chemical and Biological Controls in Forestry*, ed. W. Y. Gamer and J. Harvey, Jr. ACS Symposium Series No. 238. American Chemical Society, Washington, DC, pp. 351–63.

Wilkinson, R. E. & Smith, A. E. (1975). Thiocarbamate inhibition of fatty acid biosynthesis in isolated spinach chloroplasts. *Weed Sci.*, **23**, 100.

Yentsch, C. M., Cucci, L. & Phinney, D. A. (1984). In *Marine Phytoplankton and Productivity*, ed. O. Holm-Hansen, L. Bolis and R. Gilles, pp. 141–55.

Zsoldos, F. & Hanunold, E. (1978). Potassium influx and efflux of 2,4,-D and MCPA-treated rice plants. *Plant Soil*, **49**, 219–28.