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Fate and Effects of Salicylic Acid Compounds in Freshwater Systems

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The comparative fates and effects of salicylic acid (SA) and Na salicylate in algae (*Scenedesmus subspicatus, Monoraphidium minutum*), in *Lemna minor*, and in *Daphnia magna* were examined. Test methods were principally based on the OECD testing guidelines with modifications in the procedures. The influence of fulvic acid (FA) on bioconcentration and on toxic effects was studied. FA addition significantly reduced the bioavailability of SA in *L. minor* and the algae species. SA was more toxic to Lemnaceae, algae, and daphnids than to its Na salt. Bioconcentration factors in *S. subspicatus, M. minutum*, and *L. minor* were about 10^3 in 72–96 hr. The reproducibility of *D. magna* was reduced by 38% at a concentration of 20 mg SA/liter.

INTRODUCTION

Aromatic carboxy–hydroxy compounds are ubiquitous substances of natural and anthropogenic sources. The distribution of salicylic acid (SA), one of the most prominent chemicals of that group in soil, water, and plants, is well documented (Whitehead, 1964; Melton *et al.*, 1981; Buta, 1984; Schwarzenbach-Giranden, 1984). The fate of salicylic acid in the environment has been studied as a possible model substance for the degradation of natural polymers like humic and fulvic acids (FA) (Brunow *et al.*, 1976). The influence of, e.g., fulvic acid on the environmental toxicity of pesticides and of toxic heavy metals has become one of the actual subjects of ecotoxicological research (Cambell and Evans, 1987; Klöcking, 1980; Galbs *et al.*, 1984). SA and substances with related structure are supposed to be able to alter the toxicity of certain chemicals.

The aquatic planktonic biomass usually represents a sensitive indicator for changes in water quality. Physiological reactions and deviations from the "normal" oscillations are sensitive responses of an affected biotope for which detection systems and indicator organisms have been established. The present study focuses on the chemical fate and the toxic effects of SA and Na salicylate (NaSA) on the representative freshwater organisms green algae, Lemnaceae (duck weed), and daphnids under laboratory conditions. Fulvic acid, additionally, was applied to evaluate possible antagonistic effects on the biomass.

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MATERIALS AND METHODS

Bioconcentration of SA in Lemna minor

L. minor (duckweed) was cultured under laboratory conditions in a specific nutrient solution (recipe available upon request). For the uptake study, 10 plants each (one-leaf stage) were transferred into 2-liter beakers at 20°C and an illumination of 115 $\mu E/m^2$ sec with a day–night cycle of 12 hr. Three replicates were provided for each chemical concentration. Water evaporated from the test beakers was replenished daily with distilled water.

SA was ^{14}C-labeled in the side group and purchased from New England Nuclear (sp act 59.5 mCi/m*M*, radiochemical purity > 99%).

The chemical concentration in the water was kept below 50 μg per liter to avoid acute toxic influences upon *L. minor*. Fulvic acid extracted directly from river water was characterized physicochemically (Peng and Wang, 1981). The concentrations of FA in the test medium were 2 and 10 mg per liter, respectively. ^{14}C-SA derived residues in the tissues of *L. minor* were determined daily by combustion of aliquots to $^{14}CO_2$ in an oxidizer (Model 306, Packard Instruments). Radioactive liquid phases were quantified by liquid scintillation counting (Model 800, Berthold) using Hydroluma (Baker) as scintillation cocktail. The bioconcentration factor (BCF) was calculated from the quotient between the chemical concentration in *L. minor* (dpm/g wet wt) and the concentration of ^{14}C in water (dpm/ml).

Toxicity Test with L. minor

The parameters and conditions were as described before for the BCF study. The nutrient solution was limited to 200 ml per beaker; experimental time was 7 days. SA was applied in concentration steps of 15, 30, 60, and 120 mg per liter. The parameters recorded after terminating the experiment were *biomass* (dry weight), *chlorophyll* contents (extraction with 95% alcohol in darkness for 24 hr), extinction measurement at 665 nm, according to Einhellig *et al.* (Leather and Einhellig, 1985; Einhellig and Rasmussen, 1979), and *number of leaves* of *L. minor* per test vessel.

Bioconcentration of SA in Algae

For these studies the green algae *Scenedesmus subspicatus* and *Monoraphidium minutum* were cultured in our laboratories. Light, temperature, and ^{14}C measurement techniques were as described for the uptake study with *L. minor*. Experiments with green algae (three replicates, four times repetition of the test cycles) were started with 0.0148 g algae (dry wt)/300-ml nutrient solutions in Erlenmeyer flasks. (Recipes of the nutrient solutions for both algae species are available upon request.)

^{14}C-SA concentration was 50 μg per liter; FA was added at 2 and 10 mg per liter, respectively. Aliquots of the algae test solutions were taken daily and filtered by a 0.45-μm membrane before drying at 105°C to constant weight. ^{14}C-derived residues in algae and water phase were measured and quantified as described for *L. minor*.

Toxicity Tests with Algae

The green alga *M. minutum* was used for a growth inhibition test. The initial alga density in 100 ml of test solution was 10^4 cells/ml. Concentrations of SA were 10, 20,

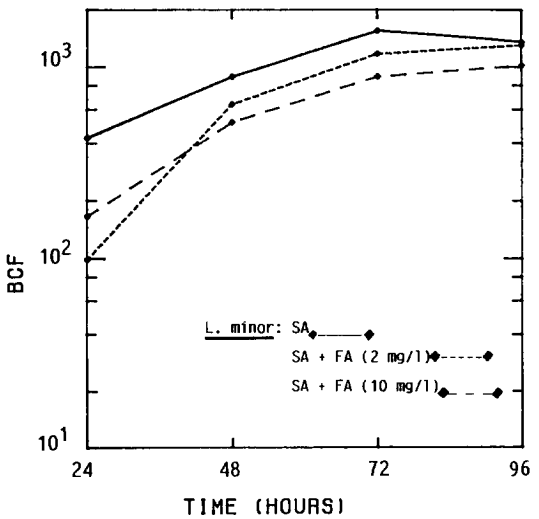

FIG. 1. Bioconcentration factors of SA in *L. minor*. Dashed curves show the influence of 2 and 10 mg FA upon the uptake of SA.

40, and 80 mg per liter, respectively. The growth inhibition was recorded daily by measuring the extinction at 660 nm (Photometer LPW, Dr. Lange). *M. minutum* was exposed to the SA concentrations for 5 days; test cycles were performed eight times.

Acute Toxicity Test with Daphnia magna

These test cycles were performed according to the OECD testing guidelines (OECD, 1984) with *D. magna* (Straus) as test organism and were repeated six times. EC_0, EC_{50}, and EC_{100} were determined for SA and for SA plus FA (10 mg per liter). EC_0 and EC_{10} were determined for NaSA and for NaSA plus FA.

Chronic Toxicity Test with D. magna

The test procedure was basically performed according to the guidelines of the OECD (1984). Temperature was 20°C, and illumination was 8 hr of darkness and 16 hr of light at 55 $\mu E/m^2$ sec. Fresh algae (*M. minutum*) were provided as a diet daily. Living and dead organisms as well as the parental and juvenile generation were counted and separated every other day. Three replicates were used for each SA concentration; the testing period lasted 22 days. A total of three test cycles was performed to obtain statistically meaningful data. If not otherwise stated, all tests with *L. minor* and the two species of green algae were repeated four times.

RESULTS AND DISCUSSION

The bioconcentration curves of SA in *L. minor, S. subspicatus,* and *M. minutum* are shown in Figs. 1 and 2. The uptake of ^{14}C-SA by *L. minor* from water phase

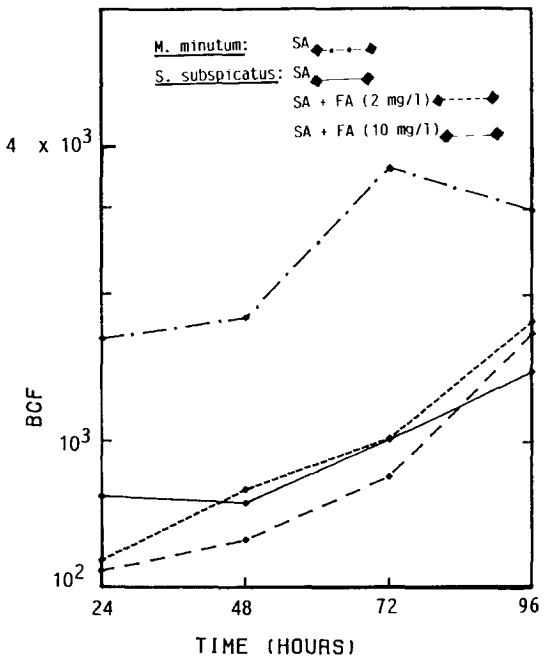

FIG. 2. Bioconcentration factors of SA in the green algae *M. minutum* and *S. subspicatus* and the influence of FA addition.

reaches a maximum after 72 hr following dosing. These findings are in agreement with previous results that demonstrated the high potential of *L. minor* to concentrate even well water soluble organic substances (Xu *et al.*, 1988). The effect of FA upon the uptake rate of SA is obvious. With the increase in FA concentration, the ^{14}C-SA derived residues were lowered (see Fig. 1, 48–96 hr). Studies on other specimens confirmed that FA reduces the bioavailability of organic and inorganic pollutants in aquatic phases (Huck *et al.*, 1987; John *et al.*, 1987). Salicylic acid is considered one of the basic model structures for the polymeric fulvic acids. Major bindings between FA and pollutants are likely by H bridges. This type of "bound residue" is blocked from passing the cell membranes of many aquatic plants because of the high molecular weight. Figure 2 illustrates the differences in bioconcentration between the two green algae species tested. *M. minutum* shows an early maximum of BCF, while *S. subspicatus* has not reached a saturation level after 96 hr of exposure to SA. Bioavailability of SA for algae was decreased for 72 hr in the presence of 10 mg FA/liter, as shown in the test with *L. minor.*

Allelopathic Behavior of Algae and Lemnaceae

The data of the toxic influences of SA and of NaSA at different concentrations are presented in Table 1. Figures 3 and 4 show the extinction curves of algae in the SA and NaSA media. SA has been observed to affect different plant developing processes (Aberg, 1981; Cleland and Ajami, 1974). The present studies demonstrate that 60 and 120 mg SA per liter consistently reduced the final leaf number, the dry weight, and the chlorophyll contents of *L. minor.* The highest dose caused a rapid yellowing

TABLE 1

EFFECTS OF SALICYLIC ACID (SA) AND Na SALICYLATE (NaSA) ON THE GROWTH, BIOMASS, AND CHLOROPHYLL CONTENTS OF *L. minor*

Conc. of SA (NaSA) (mg/liter)	No. of leaves	Biomass	Chlorophyll content	
			Tissue	Water
15	94[a]	100	102	160
	(106)	(112)	(110)	(115)
30	96	90	100	180[a]
	(100)	(100)	(95)	(100)
60	90[a]	75[a]	75[a]	220[a]
	(92)	(100)	(96)	(115)
120	20[a]	15[a]	3[a]	—
	(90)[a]	(94)	(89)[a]	(162)[a]

Note. Values represent percentage of controls after terminating the experiment. Values of NaSA are given in parentheses.

[a] Significantly different from controls.

of the leaves. NaSA only slightly inhibited the growth of *L. minor* even at 120 mg per liter concentration. The lower doses, however, had a weakly stimulating effect on *L. minor.* A similar influence was also found in a study with *L. obscura* (Leather and Einhellig, 1985).

In Table 2 the changes in the pH values of the applied media are shown. Additional toxic effects on the plants are likely at a pH below 5.

Chlorophyll contents in lemna tissues and in the ambient water are negatively correlated. This is particularly true for the test with NaSA (see Table 1). The mechanism

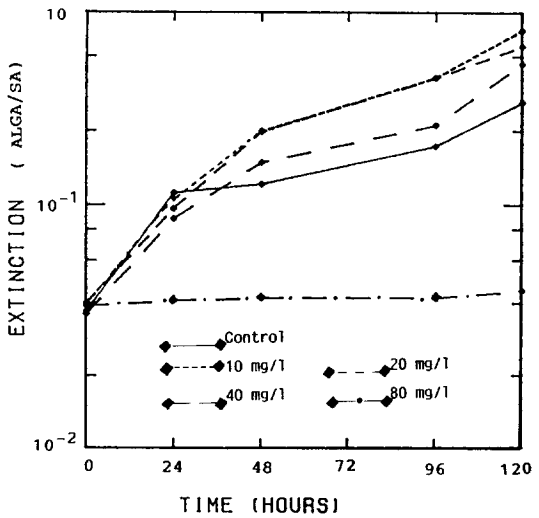

FIG. 3. Influence of SA on the growth of the green alga *M. minutum;* measured by photometric extinction.

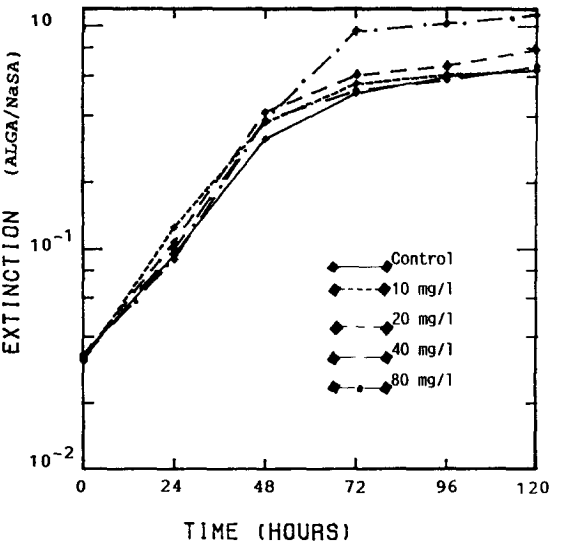

FIG. 4. Influence of Na salicylate on the growth of the green alga *M. minutum;* measured by photometric extinction.

for this kind of chlorophyll "extraction" has not been elucidated. *M. minutum* were generally stimulated in their growth at the lower NaSA concentrations (see Fig. 4). Eighty milligrams SA per liter totally inhibited the reproduction of the algae (see Fig. 3), a value which is significantly lower than the EC_{10} of SA (165 mg/liter) for the algae *Haematococcus pluviatis* (Knie *et al.*, 1983).

The strong decrease in pH was also suggested to be the major reason for the growth inhibition of algae in this test. The results of the bioavailability of SA and NaSA demonstrate that both substances are of a lower toxicity to aquatic plants in a concentration range up to 50 mg per liter and thus could be ranked into class 1 of chemicals with a low hazard potential (Geyer *et al.*, 1985).

Short- and Long-Term Toxicity Studies of SA and NaSA to D. magna

Effective concentrations of SA and NaSA to daphnids within a 24-hr testing period are shown in Table 3. FA concentrations can reduce the toxicity of both substances. At higher doses (280–320 mg/liter) secondary effects of the low pH (SA) might overlap the protective function of FA.

TABLE 2

pH VALUES OF THE DIFFERENT CONCENTRATIONS OF SA AND NaSA IN THE EXPERIMENT WITH *L. minor*

Concentrations (mg/liter):	0	15	30	60	120
pH (SA)	6.74	6.60	6.42	5.92	3.66
pH (NaSA)	6.89	6.92	7.00	7.14	7.38

Note. "0" represents the pH value of the nutrient solution for the blanks.

TABLE 3

EFFECTIVE CONCENTRATIONS OF SALICYLIC ACID (SA) AND Na SALICYLATE (NaSA) FROM ACUTE TOXICITY TESTS ON *D. magna*

EC values	SA concn.	pH	SA + FA	pH
EC_0	40	8.02	80	7.88
EC_{50}	230	6.50	235	6.40
EC_{100}	280	4.41	280	5.25
	NaSA concn.		NaSA + FA	
EC_0	80	8.68	280	8.84
EC_{10}	304	8.60	320	8.89

Note. EC values represent concentrations in milligrams per liter. Fulvic acid (FA) was added in 10 mg/liter.

In agreement with the results of *L. minor* and the green algae, NaSA is significantly less toxic than SA to *D. magna.* EC values for SA to daphnids have been determined in another study (EC_0 100 mg/liter, EC_{50} 143 mg/liter, EC_{100} 200 mg/liter) (Knie *et al.,* 1983). With respect to the general problem of the significance of a 24-hr short-term test, the data from both studies can be compared and understood as the same order of magnitude. Chronic toxicity tests are, therefore, indicated to overcome the above-mentioned insecurities. The long-term reproduction test of SA to *D. magna* yields more useful information about the toxicity to this test species.

In Fig. 5 and Table 4 decreasing natality with increasing concentration of SA can be seen. Summarizing and comparing the number of newborn daphnids in controls

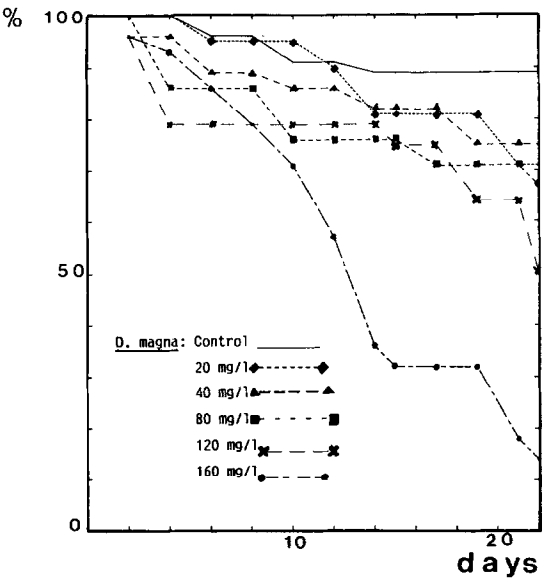

FIG. 5. Long-term chronic toxicity study of *D. magna* in different concentrations of SA, expressed as percentage surviving parental generation.

TABLE 4

CHRONIC TOXICITY (REPRODUCTION) TEST OF SALICYLIC ACID (SA) TO *D. magna*

Days:	8	10	12	14	15	17	19	21	22	8–22
Control	0	22	26	63	10	46	48	51	62	328
20 mg/liter	0	15	17	10	9	17	30	91	14	203
40 mg/liter	2	16	16	36	11	24	22	78	45	250
80 mg/liter	0	14	34	24	4	28	18	75	79	276
120 mg/liter	0	6	5	17	2	9	27	20	87	173
160 mg/liter	0	0	0	3	0	0	8	0	2	13

Note. Values represent the total number of newborn daphnids for each chemical concentration at different days, during 22 days of experiment. The last column represents the total number of individuals for each chemical concentration and the controls.

and 40-mg SA-treated vessels, one finds the reproduction capacity to be lowered by 25% in the presence of SA. Forty milligrams SA per liter, however, was found to be the no-effect level (EC_0) from the 24-hr acute test. In the long-term study, even at 20 mg/liter SA concentration, the reproduction suppression is about 38% (percentage of cumulative values on Days 14 and 22 of experiment) compared to the controls.

CONCLUSION

The present investigations into the fate and the effects of SA/NaSA clearly point out that a combination of different short- and long-term tests is needed to evaluate safe toxicity data for aquatic indicator organisms. To assess the overall environmental hazard potential, field studies under natural conditions must be conducted, especially with respect to the role of natural substances like humic acids.

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