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PHYTOTOXICITY STUDIES WITH *LACTUCA SATIVA* IN SOIL AND NUTRIENT SOLUTION

E.M. HULZEBOS,*† D.M.M. ADEMA,‡ E.M. DIRVEN-VAN BREEMEN,† L. HENZEN,‡ W.A. VAN DIS,† H.A. HERBOLD,† J.A. HOEKSTRA,† R. BAERSELMAN† and C.A.M. VAN GESTEL†
†National Institute of Public Health and Environmental Protection (RIVM), P.O. Box 1, 3720 BA Bilthoven, The Netherlands
‡TNO Institute of Environmental Sciences (TNO), P.O. Box 6011, 2600 JA Delft, The Netherlands

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Abstract—The toxicity of 76 priority pollutants to lettuce (*Lactuca sativa*) was determined in soil and in nutrient solution. In the first case a static and in the latter a semistatic exposure was established. Volatile and easily degradable compounds had high EC50 values in soil (>1,000 μg/g). In nutrient solution, however, several of these compounds were rather toxic. Quantitative structure-activity relationships (QSARs) relating EC50 values to log K_{ow} could be described for the toxicity in nutrient solution. Generally, the toxicity of the compounds increased with increasing lipophilicity. Deviations were (partly) caused by reactivity (N-containing compounds, double bonds in compounds), low lipophilicity (log K_{ow} values <1), and EC50 values close to solubility. To relate toxicity in soil and nutrient solution, soil EC50 values were recalculated to values in the soil pore water using calculated adsorption coefficients. Estimated pore-water EC50 values showed a good correlation with values determined in nutrient solution but were not equal to these values. The differences can be attributed to differences in exposure (static vs. semistatic).

Keywords—Organic chemicals Pesticides Plant growth effects Bioavailability Structure-activity relationships

INTRODUCTION

In the framework of the Dutch Chemical Substances Act, a program has been set up to determine the effects of priority pollutants on plant growth according to Organization of Economic Cooperation and Development (OECD) Guideline 208 [1]. Based on the results of the first part of this program, *Lactuca sativa* was chosen as a test species, because it was sensitive to different organic compounds and easy to handle [2]. In the second part, the phytotoxicity of 76 organic substances was determined in soil and in nutrient solution. This paper describes the results of the second part of this phytotoxicity program. Effects of pollutants in the field can be predicted on the basis of laboratory tests only if the routes of exposure are similar. To predict the effect of chemicals in soil on higher plants, it is therefore necessary that laboratory phytotoxicity tests are carried out in soil. In such soil tests, plants cannot, however, be exposed continuously. Volatile and degradable compounds disappear from the soil, and effects might be underestimated. In the soil ecosystem, plants are often exposed continuously, because pollutants will be supplied from the air and/or deeper soil layers. If tests are performed in nutrient solution, semistatic exposure can be realized, providing more information about the actual toxicity of the compound to plants. For this reason in this phytotoxicity program, besides tests in soil, all compounds were also tested in nutrient solution (with semistatic exposure). In this paper, the effects of 76 priority pollutants on the growth of *L. sativa* in soil and nutrient solution are described and the literature is reviewed. EC50 values obtained in nutrient solution are related to lipophilicity (quantitative structure-activity relationships [QSARs]). Van Gestel and Ma [3,4] demonstrated that the earthworm toxicity of organic chemicals in soil is determined mainly by the concentration in the soil pore water, and that pore-water concentrations can be predicted using adsorption coefficients. This pa-

*To whom correspondence may be addressed.
The current address of C.A.M. van Gestel is Department of Ecology and Ecotoxicology, Vrije Universiteit, De Boelelaan 1087, 1081 HV Amstelveen, The Netherlands.

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per follows their approach to investigate the relation between phytotoxicity data obtained in soil and nutrient solution.

MATERIALS AND METHODS

Test species

Lactuca sativa (lettuce) Ravel R2.

Test compounds

(Chloro)(methyl)phenols. Phenol; 2-monochlorophenol (2-MCP); 3-monochlorophenol (3-MCP); 2,4-dichlorophenol (2,4-DCP); 3,5-dichlorophenol (3,5-DCP); 2,4,6-trichlorophenol (2,4,6-TCP); 2,3,5-trichlorophenol (2,3,5-TCP); pentachlorophenol (PCP); 4-chloro-2-methylphenol (4C2MP); 4-chloro-3-methylphenol (4C3MP); catechol; β-naphthol; *o*-cresol; *m*-cresol; and nonylphenol.

(Chloro)anilines. Aniline; 2-chloroaniline (2-MCA); 3-chloroaniline (3-MCA); 2,4-dichloroaniline (2,4-DCA); 3,4-dichloroaniline (3,4-DCA); 3,5-dichloroaniline (3,5-DCA); 2,4,5-trichloroaniline (2,4,5-TCA); 2,4,6-trichloroaniline (2,4,6-TCA); 2,3,4,5-tetrachloroaniline (2,3,4,5-TeCA); 2,3,5,6-tetrachloroaniline (2,3,5,6-TeCA); and pentachloroaniline (PCA).

Chloro(nitro)benzenes. Chlorobenzene (MCB); 1,4-dichlorobenzene (1,4-DCB); 1,2,3-trichlorobenzene (1,2,3-TCB); 1,2,4-trichlorobenzene (1,2,4-TCB); 1,3,5-trichlorobenzene (1,3,5-TCB); 1,2,3,4-tetrachlorobenzene (1,2,3,4-TeCB); 1,2,4,5-tetrachlorobenzene (1,2,4,5-TeCB); pentachlorobenzene (PCB); hexachlorobenzene (HCB); 1-chloro-2-nitrobenzene (C2NB); 1-chloro-3-nitrobenzene (C3NB); and 2,3-dichloro-1-nitrobenzene (2,3-DCNB).

Miscellaneous compounds. Nitrogen-containing compounds: ethylenediamine; dipropylamine; dibutylamine; acrylamide; acetanilide; 1,2-diaminobenzene; 2,5-diaminotoluenesulfate (2,5-DATS); trypan blue; rhodamine; pyridine. (Chloro)(cyclo)alk(a)(e)nes: tetrachloroethene; hexachlorocyclopentadiene (HCCP); heptane; octane; decane; 1,1,1-trichloroethane; β-hexachlorocyclohexane (β-HCH). Aromatics: acenaphthene; toluene; naphthalene; *o*-xylene; styrene. Pesticides: azinphos methyl; bis(dimethylthiocarbamoyl)disulfide (ARASAN); triazophos; endosulfan. Phthalates: diethylphthalate (DEP); dibutylphthalate (DBP); diethylhexylphthalate (DEHP). Others: propyleneglycol; isopropylalcohol; isobutylalcohol; benzaldehyde; *n*-butylacetate; thiophene; furan, thiophenol; and biphenyl.

Test compounds were supplied by Aldrich Chemie, J.T. Baker, BDH, EGA-Chemie, Fluka A.G., Janssen, and Merck and purity was ≥95%, except for 2,4,6-TCA, which was technical grade; HCB, 74.7%; and triazophos, rhodamine, and trypan blue, which had a purity of about 80%.

Plants were cultured in climatic chambers to maintain a test temperature of 21 ± 4°C and to provide a photoperiod of 16:8 h light:dark at a light intensity of 6,500 lx under fluorescent tubes. Humidity varied between 40 and 80%; it was highest and temperature was lowest during the dark period.

Tests were carried out by two institutes, the National Institute of Public Health and Environmental Protection (RIVM), Bilthoven, The Netherlands, and the TNO Institute of Environmental Sciences (TNO), Delft, The Netherlands. A number of compounds were tested by both institutes, to check the reproducibility of the tests (see Table 1).

Soil tests

Soil was collected twice from an orchard. The characteristics of these two collections of agricultural loam differed slightly. The first collection, used by TNO, agreed with the specification of OECD Guideline 208 [1]: pH (1 N KCl) was 7.5, organic matter content 1.4%, and clay content 12%. The clay content of the second collection, used by RIVM, was twice as high as the first; the organic matter content was 1.8%; pH was the same. K_2HPO_4 had to be added to this second collection to achieve good plant growth. Soils were 4-mm-sieved before the tests started.

Test compounds were dissolved in water, added to the soil, and thoroughly mixed with a household mixer. Test compounds with low solubility (2,3,5-TCP, PCP, β-naphthol, all chloroanilines, all chloro(nitro)benzenes, phthalates, benzaldehyde, furan, alkanes, naphthalene, biphenyl, acetanilide, 1,2-diaminobenzene, triazophos, ARASAN, HCCP, acenaphthene, azinphos methyl, endosulfan, and β-HCH) were either dissolved in acetone and/or mixed with a small amount of quartz sand. The compound was mixed through the soil using the quartz sand as a carrier. Dimethyl sulfoxide (DMSO) was not used as a solvent, because in a pilot study it was shown to inhibit growth of lettuce in nutrient solution at 1 g/L.

Nominal concentrations in micrograms per gram dry soil were spaced by a factor of 3.2. Duplicate trays were used for the control and for at least three test concentrations. The 0.25-L plastic trays contained 400 g of soil at 25 to 30% moisture content (80% of the water-holding capacity). In each tray 10 seeds were sown. The trays were covered with glass plates until germination of seedlings. After removal of the glass plates, evaporation of water

was compensated daily by adding demineralized water. Only the first five germinated seedlings were required, and any additional germinated seedlings were discarded. After 7 and 14 d, shoots were harvested by cutting them off at soil level; the fresh weight of each plant was determined immediately after harvesting. Water content and pH of the soil were checked at the start and the end of the experiment.

Nutrient solution tests

The nutrient solution was described by Steiner [5]. In nutrient solution tests, PCP, nonylphenol, acenaphthene, tetrachloroethene, and ARASAN were dissolved in acetone. The highest concentrations of acetone (320 mg/L) affected root but not shoot growth. Degradation of acetone and of some phenols lowered the oxygen content of the test solution; this was, however, shown to have no effect on shoot growth. The TCAs, TeCAs, PCA, all chloro(nitro)benzenes (except MCB and CNBs), DEHP, alkanes, naphthalene, and biphenyl were dissolved in tributylalcohol.

Seeds were sown in plastic trays (0.25 L) filled with perlite that was saturated with nutrient solution containing different concentrations of the test compound. The trays were covered with glass plates during germination. After one week, five seedlings with roots longer than 3 cm were transferred to 1-L pots filled with nutrient solution and treated with the test compound. These solutions were renewed three times a week to achieve semistatic exposure. Duplicate pots were used for each test concentration and each control. Nominal concentrations were spaced by a factor of 3.2. At TNO shoots were harvested 16 d after sowing, and at RIVM after 21 d, and fresh weight was determined. Oxygen content and pH were measured every time solutions were renewed.

DBP ANALYSED @ TNO

Chemical analysis

The concentrations of most compounds were analyzed at the start of the tests to verify the added concentrations, and at the end of the test (soil) or just before the next replacement of the test solution (nutrient solution) to detect any decrease in concentration due to degradation or volatilization. In soil phenol, MCPs, DCPs, TCPs, PCP, and β-naphthol were extracted with water, toluene, hexane, or acetonitrile. Chloroanilines (soil) and chloro(nitro)benzenes (soil and nutrient solution) were extracted with toluene (for chloroanilines in a basic environment), followed by analysis of the extract with GC/flame ionization detector (FID) and/or GC/electron-capture detector (ECD). In nutrient solution the chloroanilines were determined directly with reversed-phase (RP) HPLC with UV detection. The miscellaneous compounds acetanilide, toluene, naphthalene, *o*-xylene, styrene, heptane, decane, trichloroethane, β-HCH, azinphos methyl, endosulfan, DEP, DBP, DEHP, *n*-butylacetate, and biphenyl were analyzed in soil. The same compounds and also octane, HCCP, rhodamine, trypan blue, and triazophos were analyzed in nutrient solution. Most compounds in soil were analyzed again after 14 d, except for *n*-butylacetate, decane, and toluene. In nutrient solution the analysis of DBP failed at $t = 0$ and $t = 2$ d, and of decane at $t = 2$ d. In soil, miscellaneous compounds were extracted with acetonitrile, methanol, carbon disulfide (CS_2), dichloromethane, or petroleum ether. In nutrient solution, CS_2 was used for extraction of the alkanes, and dichloromethane was used for acetanilide. For the other compounds extraction was not necessary. Analysis by RP-HPLC, HR-GC, detection by UV, FID, ECD, and/or nitrogen–phosphorus detector (NPD).

Statistics

The concentration at which growth was 50% of the control (EC50) was calculated by applying a logistic model according to Haanstra et al. [6]. EC50 was based only on harvested shoots; dead plants or ungerminated seeds were not taken into account.

Some compounds stimulated plant growth (hormesis), in which case the logistic model was not appropriate. Brain and Cousens [7], however, described an extension of the logistic model that allows an increase of the response at low concentrations. This model was used for 2,4-DCP to obtain a more accurate estimate of the EC50.

RESULTS AND DISCUSSION

Chemical analysis

In soil the analyzed concentrations of the compounds at $t = 0$ were generally in agreement with the nominal concentrations, mostly 70 to 150%. The analyzed concentrations of the chlorobenzenes at $t = 0$, however, showed greater differences from the nominal ones (mostly 15–131%). The lower values may have been caused by the high volatility of the chlorobenzenes (Henry's coefficients were often >0.1). The EC50 values for these compounds in soil may therefore have been underestimated. The analyzed concentrations of *n*-butylacetate and naphthalene in soil and in nutrient solution were <50%, as were the concentrations of toluene in soil and of heptane in nutrient solution. Heptane has a Henry's coefficient (H) of 72; therefore, the low

concentrations of this compound can be attributed to volatilization. The other exceptions have Henry's coefficients of <1; the low values at $t = 0$ of these compounds can be only partly due to volatilization. In some cases, samples might have been stored too long (some months) before analysis.

At the end of the test, the concentrations of most phenols in soil had dropped to a low value (<20% of the initial analyzed concentrations); in 3,5-DCP and 2,3,5-TCP (TNO), concentrations were still ≥50% of the initial analyzed concentrations.

The concentrations of aniline, MCAs, and DCAs had dropped to a low value (≤30%) of the initial concentration); the soil concentrations of TCA, TeCAs, and PCA were at least 30%, but generally >50% of the initial concentrations. The concentrations of 1,2,3,4-TeCB and PCB at the end of the test were ≥50% of the initial measured concentrations. The analyzed concentrations of the other chlorobenzenes had decreased to 8 and 41% of the initial ones. The concentrations of most analyzed miscellaneous compounds in soil had decreased to <10% of the initial measured concentrations, except for DEHP (40–60%), endosulfan (99%), and β-HCH (102%).

In nutrient solution, the analyzed concentrations of most compounds at $t = 0$ varied somewhat from 100% of the nominal levels (60–113%). The analyzed concentrations of the lower chlorinated phenols (MCPs and DCPs) were twice as high as the nominal concentrations. This was probably caused by the high dilution factor, which was needed for analyzing these phenols. Just before renewal of the nutrient solutions, in most tests the concentrations were still >50% of initial analyzed levels. Exceptions were *o*-cresol (<4–39%), β-naphthol (34–52%), PCP at RIVM (22%), and aniline (30%). The chlorobenzenes decreased to about half the initial levels. The analyzed miscellaneous compounds were >25% of the initial measured concentration, except for endosulfan (3%), *o*-oxylene (<1%), and styrene (<1%). It was concluded that initial concentrations generally were in agreement with nominal levels. All concentrations given in this paper are therefore nominal or are based on nominal amounts.

Effects

Results of all phytotoxicity tests are in Table 1, which also shows the log K_{ow} values for most compounds.

Chlorophenols. EC50 values, based on nominal concentrations, for the effect of chlorophenols and phenols on the growth of *L. sativa* in soil and in nutrient solution are given in Table 1. The EC50 values of the two chloromethylphenols could not be calculated using the described EC50 models, because growth was <50% inhibited at 32 μg/g soil, whereas seeds did not germinate at 100 μg/g soil. The EC50 of 3,5-DCP in nutrient solution had to be estimated because control plants had not grown properly. Nonylphenol inhibited growth of seedlings at 0.32 mg/L in nutrient solution; roots at this concentration were too short and seedlings could not be transferred to the growth experiment. Growth was, however, <50% inhibited after 21 d at 0.1 mg/L, and the EC50 had to be estimated. The reproducibility of the soil and nutrient solution tests was good, with EC50 values for phenol, 2,3,5-TCP, PCP, and β-naphthol determined by both institutes to differ less than a factor of three.

In soil, the EC50 values for the nonchlorinated phenols were higher than those for the chlorinated phenols. Generally, EC50 values of the latter decreased with increasing degree of chlorination. The EC50 values of the compounds were usually lower after two weeks than after one week, even though the compounds disappeared from the soil during the test period. As in soil, in nutrient solution the EC50 values for the chlorinated were lower than those for the nonchlorinated phenols.

Comparison with literature data. The following overview of the literature discusses only those data obtained in tests that are more or less comparable to the methods used in this study. Most studies described in the literature were carried out in water or in nutrient solution. Few experiments were performed in soil. In addition, literature on algal toxicity is reviewed and compared with our data on phytotoxicity in nutrient solution. Only those tests are evaluated in which algae are exposed during exponential growth, which means that tests should not last longer than 4 or 5 d and the initial cell density should not exceed 10^4 cells per milliliter [8]. Otherwise toxicity might be influenced by other factors.

Wang [9,10] determined the effects of phenol on biomass and root length of *Raphanus sativus*, *Abutilon theophiasti*, *Panicum milliacecum*, *L. sativa*, and *Cucumus sativa* after 4 or 5 d of exposure. EC50 values varied between 230 and 324 mg/L for biomass and between 120 and 220 mg/L for root length. Reynolds [11] exposed *L. sativa* seeds for 3 d and found an LC50 for germination of 132 mg/L. For the effect of phenol on the cell growth of *Elodea canadiensis* and *Lemna minor*, EC50 values of 235 and 169 mg/L, respectively, were found, after 9 to 12 d of exposure [12]. For the algae *Selenastrum capricornutum* and *Chlorella vulgaris*, EC50 values of 150 and 370 mg/L were determined after 4 d of exposure [13], and for *C. vulgaris* an EC50 of 466 mg/L was found after 6 h of exposure [14]. All the

Table 1. Effect of 76 priority pollutants on the growth of *Lactuca sativa* in soil and in nutrient solution, expressed as EC50 values with 95% C.I.s (all values are based on nominal concentrations)

Compound	EC50 (μg/g soil, t = 7 d)	EC50 (μg/g soil, t = 14 d)	EC50 (mg/L solution, t = 16–21 d)	Log K_{ow}
(Chloro)(methyl)phenols				
Phenol[a]	96 (72–129)	79 (58–107)	20 (8–46)	1.46
Phenol[b]	146 (119–180)	168 (145–195)	14 (7.8–24)	1.46
2-MCP[a]	52 (–)	43 (14–130)	16 (4–64)	2.15
3-MCP[a]	21 (12–37)	7 (3–15)	5.6 (0.5–59)	2.50
2,4-DCP[a]	27 (16–39)	53 (12–299)	2.4 (0.1–9.5)	3.06
3,5-DCP[b]	60 (18–195)	32 (–)	1 (–)	3.62
2,4,6-TCP[a]	19 (12–31)	16 (5–51)	1.8 (0.1–41)	3.69
2,3,5-TCP[a]	17 (8–35)	9 (8–11)	2.0 (1.0–4.0)	3.85
2,3,5-TCP[b]	8.5 (6.3–11)	8.9 (7.5–11)	0.79 (0.6–1)	3.85
PCP[a]	7 (4–15)	8 (4–15)	0.03 (0.01–0.1)	5.24
PCP[b]	2.7 (2.2–3.5)	3.2 (2.7–3.7)	0.03 (0.02–0.04)	5.24
4C2MP[a]	>32, <100	>32, <100	4.0 (2.4–6.5)	2.78
4C3MP[a]	>32, <100	>32, <100	2.3 (0.7–7)	3.10
Catechol[a]	>1,000	>1,000	5.0 (1.3–21)	0.88
β-Naphthol[b]	291 (218–388)	88 (72–107)	4.9 (3.9–6)	2.70
o-Cresol[a]	67 (52–86)	>100	23 (16–31)	1.95
m-Cresol[b]	69 (51–94)	96 (63–147)	50 (42–61)	1.96
Nonylphenol[a]	559 (331–946)	625 (502–>1,000)	>0.1, <0.32	
Chloroanilines				
Aniline[b]	49 (43–56)	56 (49–64)	7.9 (6.9–8.9)	0.90
Aniline[a]	32 (0.4–294)	33 (24–45)	17 (5.2–55)	0.90
2-MCA[b]	>32 (±50)	>32[c] (±50)	31 (27–37)	1.90
3-MCA[b]	17 (13–20)	15 (12–19)	5.9 (5.0–6.9)	1.88
2,4-DCA[b]	32 (24–43)	29 (24–36)	7.0 (5.7–8.7)	2.78
2,4-DCA[a]	24 (17–33)	>10, <32	6.9 (3.4–14)	2.78
3,4-DCA[b]	>10 (almost 10)	>10 (almost 10)	1.7 (1.5–1.9)	2.69
3,5-DCA[b]	16 (13–20)	13 (10–16)	5.0 (4.2–5.9)	2.90
2,4,5-TCA[b]	25 (18–35)	17 (15–20)	1.3 (1.2–1.5)	3.45
2,4,6-TCA[b]	27 (20–36)	23 (20–28)	3.5 (3.1–3.9)	3.52
2,3,4,5-TeCA[b]	47 (40–56)	24 (21–28)	0.39 (0.33–0.45)	3.94
2,3,5,6-TeCA[b]	64 (42–99)	16 (13–19)	0.62 (0.53–0.73)	4.10
PCA[b]	647 (333–1,255)	471 (296–751)	>S_w	
PCA[a]	>1,000	>1,000	>S_w	
Chloro(nitro)benzenes				
MCB[b]	1,000	>1,000	9.3 (7.5–12)	2.84
1,4-DCB[b]	213 (156–290)	248 (212–298)	5.1 (4.2–6.2)	3.52
1,2,3-TCB[b]	5.8 (4.5–7.4)	3.8 (3.4–4.2)	0.028 (0.022–0.036)	4.14[c]
1,2,3-TCB[a]	>1, <3.2	1 (0.2–5)	ND	4.14
1,2,4-TCB[b]	56 (43–74)	48 (41–56)	0.6 (0.53–0.69)	4.02
1,3,5-TCB[b]	115 (93–142)	123 (105–144)	2.0 (1.6–2.6)	4.15
1,3,5-TCB[a]	ND	ND	>0.32, <1	4.15
1,2,3,4-TCB[b]	67 (45–98)	32 (27–38)	0.63 (0.53–0.76)	4.64
1,2,4,5-TECB[b]	4.2 (2.5–7.3)	1.3 (1.2–1.5)	0.07 (0.06–0.09)	4.82
1,2,4,5-TECB[a]	2 (1–6)	2 (1–4)	>0.1, <1	4.82
PCB[b]	228 (93–554)	56 (39–81)	±1.0	5.17
PCB[a]	862 (76–>1,000)	±320	>S_w	5.17
HCB[b]	>1,000	>1,000	>S_w	
C2NB[b]	5.0 (4.0–6.0)	5.4 (4.7–6.2)	1.8 (1.6–2.0)	2.24
C2NB[a]	>3.2, <10	>3.2, <10	2.0 (0.2–22)	2.24
C3NB[b]	12 (10–14)	12 (11–13)	4.6 (4.2–5.1)	2.41
2,3-DCNB[a]	20 (17–25)	12 (9–17)	>0.32, <1	3.05
N compounds				
Ethylenediamine[a]	>1,000	692 (570–840)	208 (–)	−2.04
Dipropylamine[a]	383 (262–559)	370 (297–461)	>100, <320	1.67
Dibutylamine[a]	510 (383–680)	361 (294–444)	52 (–)	2.83
Acrylamide[a]	101 (60–170)	152 (124–186)	6 (0.5–68)	−0.67

continued

Table 1 continued.

Compound	EC50 (μg/g soil, t = 7 d)	EC50 (μg/g soil, t = 14 d)	EC50 (mg/L solution, t = 16–21 d)	Log K_{ow}
Acetanilide[b]	38 (28–50)	33 (27–41)	8.6 (7.5–9.9)	1.16
Acetanilide[a]	45 (37–54)	61 (35–108)	13 (8–22)	1.16
1,2-Diaminobenzene[b]	25 (19–32)	>10, <32	1.6 (1.4–1.9)	0.15
2,5-DATS[b]	>1,000	>1,000	7.5 (6.6–8.5)	
Rhodamine[b]	76 (60–97)	32 (28–37)	2.5 (2.1–2.9)	
Rhodamine[a]	104 (57–190)	43 (6–300)	4.6 (2.2–9.4)	
Trypan blue[b]	263 (196–352)	290 (250–337)	113 (79–161)	
Pyridine[b]	140 (119–165)	203 (176–234)	110 (96–125)	0.65
(Chloro)(cyclo)alka(e)nes				
Tetrachloroethene[a]	>1,000	>1,000	12 (5.0–28)	3.40
HCCP[a]	23 (9–62)	10 (6–18)	0.1 (0.02–0.6)	5.04
Heptane[b]	>1,000	>1,000	1.7 (1.4–2.0)	4.40
Heptane[a]	>1,000	>1,000	47 (38–65)	4.40
Octane[b]	>1,000	>1,000	>S_w	
Decane[b]	>1,000	>1,000	>S_w	
Trichloroethane[a]	>1,000	>1,000	104 (63–174)	2.49
β-HCH[a]	>1,000	>1,000	>3.2, <10	3.61
Aromatics				
Acenaphthene[a]	37 (10–133)	25 (16–40)	>0.1, <0.32	3.92
Toluene[b]	>1,000	>1,000	16 (13–21)	2.73
Naphthalene[b]	±100	>100	13 (4.8–34)	3.30
o-Xylene[a]	>1,000	>1,000	>1, <3.2	3.12
Styrene[a]	>320	>320	18 (12–26)	2.95
Pesticides				
Azinphos methyl[b]	>1,000	360 (282–459)	18 (15–19)	2.75
Azinphos methyl[a]	571 (8–>1,000)	±320	±S_w	2.75
Endosulfan[b]	>1,000	>1,000	>S_w	
Endosulfan[a]	>1,000	>1,000	>S_w	
Triazophos[b]	224 (157–318)	85 (73–99)	25 (21–30)	3.55
ARASAN[a]	>32, <100	54 (44–67)	1.2 (0.7–2)	
Phthalates				
DEP[b]	106 (89–127)	134 (116–155)	25 (21–28)	2.47
DBP[b]	387 (262–570)	>1,000	>S_w	
DEHP[b]	>1,000	>1,000	>S_w	
Alcohols				
Propyleneglycol[a]	>1,000	>1,000	56 (23–140)	−0.92
Isopropylalcohol[a]	>1,000	>1,000	>32, <100	0.05
Isobutylalcohol[b]	351 (311–396)	366 (335–400)	42 (32–54)	0.76
Isobutylalcohol[a]	873 (612–>1,000)	>1,000	15 (3–73)	0.76
Rest				
Benzaldehyde[b]	563 (495–640)	624 (549–709)	29 (25–34)	1.48
Benzaldehyde[a]	292 (144–592)	448 (268–749)	26 (8–87)	1.48
n-Butylacetate[b]	>1,000	>1,000	70 (49–100)	1.73
Furan[b]	863 (735–1,012)	617 (549–694)	130 (110–153)	1.34
Furan[a]	>1,000	>1,000	135 (−)	1.34
Biphenyl[b]	54 (39–76)	68 (56–83)	2.1 (1.3–3.3)	4.09
Thiophenol[a]	324 (219–417)	198 (124–317)	ND	
Thiophene[a]	>1,000	>1,000	42 (18–99)	1.81

(−), confidence limits could not be calculated.
S_w = water solubility.
ND = not determined.
[a]Tested at RIVM.
[b]Tested at TNO.
[c]No emergence of plants at the next higher concentration (100 μg/g); EC50 is therefore estimated to be about 50 μg/g.

EC50 values for the effect of phenol on higher plants and algae reported in the literature are approximately 10 to 20 times higher than the EC50s for lettuce reported in this study (Table 1).

Wang [9,10,15] determined effects of 2-MCP for the same plants as mentioned for phenols. EC50 values varied from 144 to 247 mg/L for biomass and from 55 to 75 mg/L for root length, except for *Lactuca sativa*, which was 130 mg/L. From these EC50 values, it may be concluded that root length is a more sensitive parameter than biomass [9,10, 15,16]. For *S. subspicatum* and *S. capricornutum*, EC50 values of 50 and 70 mg/L, respectively, were determined [13,17] after 4 d of exposure; for *C. vulgaris* values were 170 and 97 mg/L after 4 d and 6 h of exposure, respectively [13,14]. If the literature values on 2-MCP are compared with the EC50s found in this study (Table 1), they are three to 10 times higher. For 3-MCP only EC50 values for *S. capricornutum* and *C. vulgaris* were available: 29 and 31 mg/L, respectively, after 4 d of exposure [13,14]. Literature values for the toxicity of 3-MCP for algae and the EC50 found for *L. sativa* in this study are less than a factor of six.

Wang [15] and Audus and Shipton [18] determined EC50 values of 2,4-DCP for root length for *L. sativa*, *Cucumis sativa*, *Panicum milliacecum*, and *Lepidium sativum* that were 25 to 50 mg/L after 5 d of exposure. For *Linum sativum* an EC50 of 12 mg/L was determined for root length after only 19 h of exposure [19]. For the algae *S. capricornutum* and *Chlorella vulgaris*, EC50 values of 14 and 9.2 mg/L, respectively, were determined after 4 d of exposure [13]. For 3,5-DCP an EC50 value of 2.3 mg/L was determined for *S. capricornutum* [13].

The EC50s for the effect of dichlorophenols on algae are more similar to those determined in this study for lettuce (Table 1) than are the values for higher plants reported in the literature. Differences between data found in the literature and the values reported here are a factor of one to six for algae and a factor of five to 20 for plants.

The EC50 values of Wang [15] for the phytotoxicity of 2,4,6-TCP mentioned above (14–40 mg/L) were almost the same as those for 2,4-DCP. In a study by Adema and Henzen [2], EC50 values of 2,4,6-TCP for shoot biomass of *L. sativa*, *Lycopersicum esculentum*, and *Avena sativa* of 2.2, 1, and 6.8 mg/L, respectively, were determined after 19 d of exposure. For *S. capricornutum* and *C. vulgaris*, EC50 values of 3.5 and 10 mg/L, respectively, were determined [13].

Adema and Henzen [2] performed tests with 2,4,6-TCP in two different soils. Test methods were comparable to those used in this study. They found EC50 values of 144, 398, and 514 μg/g dry soil for *Lactuca sativa*, *Lycopersicum esculentum*, and *A. sativa*, respectively, for a soil containing 3.7% organic matter. The soil used in this study contained 1.4% organic matter. Assuming that organic compounds will adsorb mainly on soil organic matter [20], the EC50 values can be compared with those in Table 1 by correction for the difference in organic matter content. After this correction Adema and Henzen's [2] EC50 for *Lactuca sativa* is still higher than that found in this study (16 μg/g), but the difference is less than a factor of 3.5. Adema and Henzen [2] also tested the three plant species in the same soil type as that used in this study but found approximately the same EC50 values as those in the soil with 3.7% organic matter. No explanation for this difference was found.

All the EC50 values reported in the literature on the toxicity of 2,4,6-TCP for plants and algae are of the same order of magnitude as those found for lettuce in this study (Table 1).

Wang [15] determined EC50 values of 5, 11, and 40 mg/L for the effect of PCP on the root length of *L. sativa*, *P. milliacecum*, and *Cucumis sativa*, respectively, after 5 d of exposure. For *Brassica rapa* an EC50 value of 1.76 mg/L was found for shoot biomass after 3 d of exposure [21]. For the inhibition of cell growth of *Elodea canadiensis* and *Lemna minor*, EC50 values of 1.4 to 0.4 and >1.4, respectively, were determined after 7 to 14 d of exposure [22]. EC50 values for *S. capricornutum* varied between 0.19 and 0.4 mg/L [23–25]; for *Scenedesmus quadricaudum*, *Chlorella pyrenoidosa*, and *C. ovalis*, these values were 0.08, 7, and 5.5 mg/L, respectively [26].

Except for *S. quadricauda*, all the EC50 values for the effect of PCP on plants and algae reported in the literature are more than a factor of 10 higher than the EC50 found for *Lactuca sativa* in this study (Table 1).

Casterline et al. [27] determined the effect of PCP on soybean and spinach in a soil containing 2% organic matter. The estimated EC50 in their study is 10 μg/g. In a similar study, Günther and Pestemer [21] determined EC50s for *B. rapa* and *A. sativa* of 10 to 20 μg/g, using a sandy loam soil (pH 6.1; 2.2% organic matter). These values are quite similar to the value found in this study (3.2 μg/g), especially when the higher soil organic matter content in these studies is taken into consideration.

Megharaj et al. [28] determined the effect of catechol on the alga *C. vulgaris* in a soil contain-

ing 1.8% organic matter. The estimated EC50 after 10 d was >50 μg/g, but after 20 d it was 20 to 50 μg/g, which is much lower than the value found in this study (>1,000 μg/g). Possibly catechol disappeared more rapidly from the soil of this study than from that of Megharaj et al. [28], who used a closed system. The EC50 of 10 mg/L for the toxicity of catechol in nutrient solution reported by Megharaj et al. [28] for *C. vulgaris* is much more similar to the value found for *L. sativa* in this study (5 mg/L). The LC50 values of 71.6 and 28.8 mg/L, respectively, for the effect of catechol and β-naphthol on the germination of *L. sativa* in nutrient solution reported by Reynolds [29] are much higher than the EC50 values found in this study.

The effect concentrations reported in the literature for the effect of catechol and β-naphthol on higher plants are higher than those found in this study.

Generally, the EC50 values for the effect of phenols on higher plants reported in the literature are approximately 10 times higher than those found in this study. In many cases this can be explained by the longer exposure period used in this study. In the study of Aberg [19], after only 19 h of exposure to 2,4-DCP root length was, however, as much inhibited as in the other studies. As indicated by Wang [9,10,15,16], root length seems to be a more sensitive parameter than biomass. Sund and Nomura [30] exposed seeds of *R. sativus* and *Sorghum bicolor* for only 1 d to chlorophenols, and LC50s for seed germination were of the same order as those found in the other studies described here. Chlorophenols probably affect the oxidative phosphorylation, a basic step in respiration [31]. Even a short exposure to chlorophenols may inhibit the first process in germination, and seeds might be killed. In this study, chlorophenols also inhibited germination abruptly above 100 μg/g or mg/L, and germination did not recover after test compounds had disappeared.

The EC50s for algae are generally in agreement with those for higher plants found in the literature. For some compounds (catechol, DCPs), algae are as sensitive as lettuce in this study.

Differences between species can be determined within only one test method. In root elongation tests, *P. milliacecum* seems to be the most sensitive [9,10,15,16]. For germination tests [11,29] and shoot biomass tests [2], *L. sativa* is shown to be a sensitive species. Aquatic plants, like *Lemna* and *Elodea*, seem to be less sensitive [12,22]. In algal tests *Scenedesmus* and *Selenastrum* are usually more sensitive than *Chlorella*. Blue-green algae seem to be more sensitive than green algae but are not included in this review [32,33].

Chloroanilines. For aquatic organisms the toxicity of chloroanilines increases with log K_{ow} or number of chlorine atoms [34]. The results of the experiments in nutrient solution reported here (Table 1) also broadly show an increase of toxicity for lettuce with increasing chlorine number, although the difference between isomers is sometimes quite large. 3-MCA is more toxic than 2-MCA, and 3,4-DCA is more toxic than the 2,4- and 3,5- isomers. Proper testing of PCA was difficult due to its low water solubility.

Differences between effect concentrations in soil were found to be small for the different chloroanilines (Table 1). With PCA, however, very high concentrations were necessary to obtain effects; at RIVM the EC50 value was not reached at the maximum tested concentration.

Three compounds were tested in both laboratories: aniline, 2,4-DCA, and PCA. As stated above, proper testing of PCA was difficult as a result of its low water solubility. Similar to the findings on chlorophenols, the results for aniline and 2,4-DCA indicated a good reproducibility between the two laboratories.

Comparison with literature data. For higher terrestrial plants, Chrysostom [35] determined the effects of aniline on root growth of 3-d-old seedlings of *Lupinus albus* in nutrient solution. The EC50 value, 18.6 mg/L, was about the same as that found in this investigation for shoot growth (7.9–17 mg/L).

Adema and Henzen [2] tested 2,4-DCA in three higher plants (*Lactuca sativa*, *Lycopersicum esculentum*, and *A. sativa*) in two types of soil and in nutrient solution. One of the soil types was the same as that used in this study. The results for this soil type and for the nutrient solution were about the same in both investigations.

For aquatic plants, Adema [36] reported an EC50 for *Lemna minor* (duckweed) of 10 mg/L for 2,4-DCA, which is about the same as our finding for lettuce in nutrient solution (6.9–7.0 mg/L).

For green algae, EC50 values of aniline for cell multiplication during exposure times of 2 to 8 d are in the range of 8 to 50 mg/L [32,37–39]. These EC50 values for aniline are in good agreement with those for higher plants reported in Table 1 (7.9–17 mg/L in nutrient solution).

Adema [36] reported EC50 values of about 10 mg/L for 2,4-DCA in three different green algae. These data are in agreement with those for duckweed and the three higher plants in nutrient solution as well as the data reported here.

For 3,4-DCA, EC50 values of 2 to 3 mg/L were found for two different green algae species (*C. vulgaris* and *S. quadricauda*) [26]; these values are in agreement with the value found in this investigation for lettuce in nutrient solution (1.7 mg/L).

In general, it can be concluded that the toxicity of (chloro)anilines for green algae, duckweed, or higher plants is about the same under comparable test conditions.

Chloro(nitro)benzenes. The EC50 of MCB in soil was ≥1,000 μg/g; it is a volatile compound, and probably seeds were hardly exposed. The EC50 value of HCB was >1,000 μg/g; because this compound is hardly soluble, the amount present in the soil pore water was probably too small to cause 50% growth inhibition. TCBs and TeCBs had lower EC50 values than the other chlorobenzenes. The differences within the groups of TCBs and TeCBs were larger than those between these two groups. Especially the EC50 values of 1,2,3-TCB and 1,2,4,5-TeCB were rather low compared to the EC50 values of the other tested chemicals.

In nutrient solution the ranking of compound toxicity was comparable with that in the soil. The EC50 value of MCB was highest and that of 1,2,3-TCB the lowest. Also, in nutrient solution the EC50 value of PCB was lower than those of TCBs and TeCBs. The different isomers of TCB differed considerably in toxicity: EC50 for 1,2,3-TCB was a factor of 200 lower than that for 1,3,5-TCB. Toxicity of TeCBs was of the same order as that of the TCBs. A nitrogen atom seems to have the same inhibitory effect as a chlorine atom: both CNBs were as toxic as DCB, and the EC50 of 2,3-DCNB was in the same range as that of the TCBs (Table 1). The EC50 of 2,3-DCNB had to be estimated; at 1 mg/L the roots of the seedlings were too small to transfer them from the preculture on perlite to the nutrient solution, but at 0.32 mg/L growth was <50% inhibited.

Wong et al. [40] found an increasing toxicity with increasing degree of chlorination. In our study the number of chlorine atoms is not the only determining factor for toxicity, neither in soil nor in nutrient solution. Chloro(nitro)benzenes are volatile, which decreases exposure, especially in soil. From the results of chemical analysis, it could be concluded that 1,3,5-TCB disappeared about twice as fast from soil as 1,2,3-TCB. This, however, cannot fully explain the difference between the EC50 values of the two. The higher toxicity of 1,2,4,5-TeCB compared with that of 1,2,3,4-TeCB also cannot be explained, as they have about the same lipophilicity (Table 1). The dissipation of the latter was much slower than that of 1,2,4,5-TeCB, so the higher toxicity of the 1,2,4,5-TeCB is not the result of differences in exposure. Besides, in nutrient solution the same differences are found between the isomers of TCBs and TeCBs.

It seems that other factors also play an important role in the toxicity of some chlorobenzenes. Williams et al. [41] found that the extent of metabolism of chlorinated benzenes and potential epoxy formation depended on the number of unsubstituted free vicinal carbon atoms. A higher degree of reactivity and metabolism might increase toxicity, due to the more reactive intermediates formed. Based on the reactivity of the free vicinal carbon atoms within the group of the TCBs, the expected order of toxicity is 1,2,3-TCB > 1,2,4-TCB > 1,3,5-TCB [41]. Sicko-Goad et al. [42] have determined the effects of chlorobenzenes on the fatty acid composition of diatoms. As 1,2,3,4-TeCB has two free vicinal carbon atoms and 1,2,4,5-TeCB has none, the first was expected to be more toxic than the latter. Comparing these predictions with our data, it can be seen that TCB toxicity increases with increasing number of free vicinal carbon atoms. 1,2,4,5-TeCB toxicity is, however, much higher than the value predicted from its free vicinal carbon atoms (none). Ameen et al. [43] found a high toxicity for 1,2,4,5-TeCB. In the sixties, 1,2,4,5-TeCB was used to control wild oats. It might have a special phytotoxic action that cannot be predicted on the basis of its free vicinal carbon atoms. PCB is less toxic than could be expected from its lipophilicity and volatility, but as it has no free vicinal carbon atoms, its reactivity is low [41].

Comparison with literature data. Effects of chlorobenzenes on plant growth are hardly reported in the literature. Ameen et al. [43] determined the effect of 1,2,4,5-TeCB on germination and growth of oats and barley in four soils differing in clay and organic matter content. Seeds were sown 1 d after mixing 1,2,4,5-TeCB through the top layer of the soil. In the sandy soil no germination and growth occurred at ≥2.5 μg/g. Only in the clayey soil did seedlings develop, and EC50 for seedling heights in this soil was 10 μg/g for barley and 3 μg/g for oats. In a sandy soil germination and growth were comparable with the control only when seeds were sown 125 d after treatment at 200 μg/g. These estimated EC50 values are in agreement with the EC50 value found in this study.

EC50 values of MCB for the algae *Ankistrodesmus falcatus* and *Selenastrum capricornutum* were 50 and 12.5 mg/L, respectively; for 1,4-DCB, 20 and 1.6 mg/L, respectively; for 1,2,3-TCP, 6 and

0.9 mg/L, respectively; and for 1,2,4-TCB, 6 and 1.4 mg/L, respectively [40,44]. EC50 values of 1,3,5-TCB, 1,2,3,4-TeCB, 1,2,4,5-TeCB, and PCB were determined only for *A. falcatus*: 9.1, 4.1, 5.0, and 1.3, respectively [40]. EC50 values of C2NB, C3NB, and 2,3-DCNB were determined for *C. pyrenoidosa*: 6.9, 1.9, and 2.9, respectively [45]. The EC50 values for *A. falcatus* [40] were approximately five times higher than those found in this study. For *S. capricornutum* [44] the differences were even smaller. In addition, the EC50 values of the (D)CNBs for algae [45] were in good agreement with those for *Lactuca sativa*. There were only two exceptions: 1,2,3-TCB and 1,2,4,5-TeCB. The toxicity of these two compounds for *L. sativa* was high, compared to the other isomers and literature data. In a 48-h test with *Scenedesmus subspicatum*, the EC50 values for 1,4-DCB and C2NB were 28 and 34 mg/L, respectively [17]. These values were quite high, compared to those found in other algal studies and in this study.

Miscellaneous compounds. In soil most of the N compounds had EC50 values between 100 and 1,000 μg/g; acetanilide, 1,2-diaminobenzene, and rhodamine had lower EC50 values: 10 to 100 μg/g. In nutrient solution the same trend was found, but acrylamide and especially 2,5-DATS were relatively more toxic.

The toxicity of the N compounds in nutrient solution varied highly, especially in regard to their lipophilicity (expressed in log K_{ow} values; Table 1). For instance, acrylamide with a low log K_{ow} value was quite toxic (EC50 6 mg/L). For 1,2-diaminobenzene and 2,5-DATS, the two amino groups of the molecule might have caused the relatively high toxicity. Rhodamine was quite toxic to plants (EC50 2.5 mg/L), even though it was readily soluble (log K_{ow} was not known).

Reynolds [46,47] determined the inhibition of the germination of lettuce seeds by some amines after 3 d of exposure in aqueous solutions: LC50 values were 1,239 mg/L for dipropylamine, 1,021 mg/L for *n*-butylamine, 406 mg/L for acetanilide, and 158 mg/L for pyridine. The EC50 values in our study were lower, probably because seeds and plants were exposed for a longer period. For diisopropylamine, phytotoxicity was assessed by Adema and Henzen [2] in a pilot study. EC50 values for lettuce, tomato, and oats were 560, 624, and 725 μg/g, respectively, in loam (pH 7.5, organic matter content 1.4%) and 953, 1,118, and 1,880 μg/g, respectively, in humic sand (pH 5.1, organic matter content 3.7%). These EC50 values are in the same range as the ones found for dipropylamine in this study, which used the same loam. In humic sand the values were about three times higher, which can be explained by its higher organic matter content. The EC50 values for lettuce, tomato, and oats in nutrient solution were 283, 180, and 594 mg/L, respectively; these values are also in the same range as that found for dipropylamine in this study (>100–<320 mg/L).

Other data about amines were found in algal studies. Calamari et al. [38] determined EC50 values for dipropylamine and dibutylamine of 20 and 19 mg/L after exposing *Selenastrum capricornutum* for 96 h. The EC50 for dipropylamine was 10 times lower than that found in this study. Kühn and Pattard [17] determined an EC50 for ethylenediamine >100 mg/L after exposing *S. subspicatum* for 48 h.

In soil, in the group of the (chloro)(cyclo)alk(a)(e)nes, an EC50 value could be determined only for HCCP. For all other compounds, the EC50 was >1,000 μg/g. Most of the (chloro)(cyclo)alk(a)(e)nes, such as heptane, tetrachloroethene, and trichloroethane, are very volatile ($H > 0.1$), which may have resulted in a rapid loss from test substrates. It is therefore likely that seeds and seedlings in soil were hardly exposed to these compounds, explaining the high EC50 values.

In nutrient solution the EC50 values of octane and decane were higher than their water solubilities. Low EC50 values (≤12 mg/L) were determined for the other compounds, except for trichloroethane (EC50 104 mg/L). In the semistatic nutrient solution, the EC50 for heptane was already reached at 1.7 mg/L. The volatilization of heptane from nutrient solution at RIVM was quite fast: at $t = 0$ the analyzed concentration was approximately 50% of the nominal value, and after 2 d only about 20% was left. This might also explain the large difference in EC50 values between TNO and RIVM: 1.7 and 47 mg/L, respectively. For 1,1,2-trichloroethane an EC50 of 170 mg/L was found for *C. pyrenoidosa* (96 h) [26], which is in the same range as the EC50 for 1,1,1-trichloroethane for lettuce in this study (104 mg/L).

In soil the EC50 values of most aromatics were higher than the highest tested concentration, whereas in nutrient solution EC50 values of all aromatics remained below 20 mg/L. Acenaphthene had the lowest EC50 in soil and in nutrient solution.

Reynolds [46] determined an LC50 value greater than solubility for naphthalene in his lettuce seed germination test after 3 d of exposure. In our study the EC50 was two to three times lower than its solubility. For toluene in two algal studies with *S. capricornutum* the EC50 was 9.4 and 12.5 mg/L,

respectively, and for *o*-xylene 4.2 and 4.7 mg/L, respectively [48,49]. These values are in the same range as those found in this study for lettuce.

Among the pesticides, ARASAN and triazophos had the lowest EC50 values in soil; for endosulfan EC50 values were higher than the highest tested concentration. In nutrient solution ARASAN was the most toxic compound. The EC50 of endosulfan was higher than its water solubility. The EC50 values of the other compounds were in the same range (20–40 mg/L).

In two field studies, endosulfan was more toxic to plants and algae than it was in this study. Endosulfan inhibited germination of lucerne, bean, and sorghum with 4.5 to 31.2% at 5 mg active ingredient per kilogram (a.i./kg) [50]. Growth of soil algae was approximately 50% inhibited when endosulfan was applied at 100 mg a.i./kg to a nonflooded red laterite soil (pH 8.7, organic matter content 2%) [51]. Endosulfan was applied as an emulsifiable concentrate in the latter study and possibly also in the first, which might have increased bioavailability.

The phytotoxicity data on azinphos methyl found in the literature by far exceed the water solubility of this compound. Germination of *Vicia faba* was approximately 50% inhibited at 1,000 to 2,000 mg/L [52].

ARASAN inhibited germination of *Beta vulgaris* by 0 to 30% during the first 4 d at 3,600 μg/g in sand or potting soil [53]. The EC50 value for ARASAN in our study was much lower (54 μg/g).

In soil and nutrient solution, DEP had lower EC50 values than the other two phthalates (DBP and DEHP). The EC50 values of DEP and DBP in soil increased during the test period, because plants recovered after initial exposure. In nutrient solution the EC50 values of DBP and DEHP exceeded water solubility. DEP, DBP, and DEHP inhibited germination of peas and spinach 40 to 50% and 25 to 50%, respectively, at 1,000 mg/L after 13 d of exposure in a static test [54]. The EC50 value for DEP in our study is much lower. Apparently, growth is a more sensitive parameter than germination, especially if percentage of germination is recorded a long time after control seeds have germinated. In our study, seeds and plants were exposed semistatically, which resulted in lower toxicity values. In a sterilized potting soil, DEP inhibited growth of peas and spinach for 20 to 25% after 14 to 16 d at a concentration of 1 μg/g. Whenever *Micrococcus* was added, growth of seedlings was similar to that of the control [54]. In our study the effect concentration was higher (EC50 134 μg/g), for which no explanation could be found. In a study of Shea et al. [55], the effect of DBP on the growth of *Zea mais* was determined in soil (organic matter 1%, pH adjusted to 6.0) after three weeks. Germination was not inhibited at the highest concentration of 20,000 μg/g. EC50 value, based on shoot fresh weight, was 2,000 to 20,000 μg/g. This high EC50 value was probably caused by the duration of the test period. In our study the EC50 after 14 d was much higher than after 7 d (134 and >1,000 μg/g, respectively). Apparently plants are able to recover after dissipation of these compounds from the soil.

The EC50 values of the alcohols in soil were 100 to >1,000 μg/g. In nutrient solution their EC50 values were in the range of 10 to 100 mg/L. The effect concentrations of propyleneglycol, isopropylalcohol, and isobutylalcohol on germination of lettuce seeds [11], root growth of wheat [56,57], root growth of lupine [58], and growth of the algae *Scenedesmus subspicatum* [17] in aqueous or nutrient solutions were all far above 1,000 mg/L, after a test period of 1 to 7 d. In this study much lower EC50 values were determined, probably a result of the longer test period.

The EC50 values of the compounds in the rest of the group in soil were between 100 and 1,000 μg/g, except for biphenyl (EC50 68 μg/g). In nutrient solution, biphenyl was also the most toxic compound (EC50 2.1 mg/L) and furan the least (EC50 130 mg/L). Reynolds [11,46] determined the effect of benzaldehyde and furan on the germination of lettuce seeds after 3 d: LC50s were 66.3 and 558 mg/L, respectively. These values are higher than the EC50 values found in our study, probably due to the longer test period.

Conclusion

In general, the EC50 values in soil decreased rather than increased between days 7 and 14 of exposure. Increasing EC50 values are expected when compounds dissipate rapidly from the soil, enabling plants to recover. The EC50 values of the analyzed compounds naphthalene, two of the phthalates, and benzaldehyde increased during the test period. These compounds could not be detected at the end of the test period, indicating that the increase of EC50 values was due to decreasing exposure levels. For a number of compounds, like alkanes and some of the aromatics, the EC50 values in soil were >1,000 μg/g. Compounds with Henry's coefficients >0.1 are hardly effective in soil tests because rapid volatilization leads to a fast drop in exposure concentrations.

The reproducibility of the soil and nutrient tests between the two institutes (TNO and RIVM) was good. The difference between EC50 values found in the literature and those reported in this paper could often be explained by the longer test period or the semistatic exposure we used. The EC50 values in algal studies lasting 96 h usually agreed with the EC50 values in this study. Similar EC50 values in soil in the literature were found only in the soil tests described by Adema and Henzen [2], who used the same test methods. The differences in exposure seemed to be more important than differences between species. The differences between species were usually within one order of magnitude if the same method was used. Using literature data of plants derived from different methods for extrapolation purposes, variation between species, but especially exposure concentrations and exposure time, should be taken into consideration.

QSARs

EC50 values of the compounds and octanol/water partition coefficients (log K_{ow}) derived from the CLOGP data base of the EPA are given in Table 1. No log K_{ow} was found for nonylphenol, rhodamine, trypan blue, 2,5-DATS, and ARASAN. These compounds, and those for which the EC50 exceeded water solubility, could not be used to establish a relationship between EC50 values and log K_{ow}. The difference between EC50 values determined by both institutes was usually within a factor of two to three. Therefore, in calculating QSARs, results of both institutes were used, so that in these relationships some compounds may occur twice. The relationship between the EC50 values of the different groups of compounds and their log K_{ow} values were derived by linear regression, and the results are shown in Table 2.

Phenolic compounds may dissociate in water, depending on their pK_a and the pH of the nutrient solution. Dissociated compounds are less lipophilic and exhibit a much lower toxicity than their nondissociated forms [59]. After correction for this dissociation effect by conversion of the EC50s of the phenols to the concentration of the nondissociated form, the regression line between toxicity and lipophilicity of the nondissociated phenols becomes steeper, especially if catechol with log $K_{ow} < 1$ is left out:

$$\log EC50_{nd}\ (\mu mol/L) = -1.29 \cdot \log K_{ow} + 4.93$$
$$(r = -0.94;\ n = 17)$$

The toxicity for *L. sativa* of the (chloro)anilines is well correlated with lipophilicity (Table 2). The regression and correlation coefficients increase if aniline, with a log $K_{ow} < 1$, is left out:

$$\log EC50\ (\mu mol/L) = -0.74 \cdot \log K_{ow} + 3.48$$
$$(r = -0.88;\ n = 10)$$

The effect levels of the chloro(nitro)benzenes on the growth of *L. sativa* are poorly correlated with the lipophilicity of these compounds (Table 2). Deviations are caused mainly by 1,2,3-trichlorobenzene, 1,2,4,5-tetrachlorobenzene, and PCB.

The EC50 values of the miscellaneous compounds are poorly correlated with log K_{ow} values. The fit is somewhat better when compounds with log $K_{ow} < 1$ are left out:

$$\log EC50\ (\mu mol/L) = -0.56 \cdot \log K_{ow} + 3.54$$
$$(r = -0.70;\ n = 25)$$

Most of these compounds contain one or more nitrogen groups, which might increase reactivity and therefore show high variation in toxicity [59]. In HCCP the high toxicity might be caused by the double bond, which also increases reactivity [59]. The deviation of acenaphthene and dibutylphthalate from the regression line might be caused by EC50 values close to their solubility limit.

Relationships between toxic effects on higher plants and lipophilicity of the test chemicals have hardly been reported in the literature. Reynolds [11,29,46,60] has determined LC50s for the effects on the germination of lettuce seeds for a great number of alcohols, aldehydes, aromatic compounds, carboxylic acids, and others, and related toxicity with lipophilicity, methylation, and substitution of the test compounds. These relations often show low correlation coefficients caused partly by the high variation in toxicity of compounds having low log K_{ow} values (<1), as observed in this study for

Table 2. QSARs for the relationships between log EC50 (y) for the effect on *Lactuca sativa* (in μmol/L) and the log K_{ow} (x) of phenols, chloroanilines, chlorobenzenes, miscellaneous compounds, and all compounds with log $K_{ow} > 1$

Compounds	Regression line	r	n
Phenols	$y = -0.76x + 3.50$	−0.91	18
Chloroanilines	$y = -0.54x + 2.83$	−0.86	12
Chloro(nitro)benzenes	$y = -0.46x + 2.38$	−0.61	14
Miscellaneous	$y = -0.33x + 2.83$	−0.60	33
All compounds with log $K_{ow} > 1$	$y = -0.79x + 3.63$	−0.79	65

the miscellaneous compounds. The heterogeneity of these groups also causes lower regression coefficients than those usually found with structurally related groups.

More work has been done on algae. QSARs have been reported for growth inhibition by monosubstituted phenols on *C. vulgaris* [14] and by chlorophenols on *Selenastrum capricornutum* and *C. vulgaris* [13]. The regression lines found in the latter study on *S. capricornutum* were very similar to the line we found, and this similarity increased even more when exactly the same compounds were chosen. Two growth inhibition studies on algae for chloro(nitro)benzenes using *C. pyrenoidosa* and *C. vulgaris* [38,45] were found. In this study too few chloronitrobenzenes were studied to compare the regression lines with those determined by Deneer et al. [45]. The regression line determined by Calamari et al. [38] for five low-chlorinated benzenes differed largely from that found in this study. This difference is explained partly by the higher chlorinated benzenes used in our study, but also by the relatively low EC50 value of 1,2,3-TCB. Whenever these compounds are left out, almost the same equations are found.

Lipophilicity seems to predict toxic effects best [13]; extremely lipophilic or very electrophilic compounds may, however, have an additional toxic effect [14]. If other physicochemical properties than lipophilicity are included in the QSAR equations, the relations often fit better [13,14,45,48].

From the results described in this paper, it can be concluded that the development of QSARs relating phytotoxicity in nutrient solution tests with lipophilicity ($\log K_{ow}$) seems to offer promising opportunities.

Relation between EC50 values in soil and in nutrient solution

Effect levels determined in nutrient solution cannot directly be related to soil concentrations. Apart from degradation and volatilization, lipophilic compounds may disappear from the soil pore water by adsorption, mainly to the soil organic matter [20]. The adsorbed fraction is probably not available for soil organisms [61]. Van Gestel and Ma [3,4] and Van Gestel et al. [62] have shown that the toxicity of chlorophenols, dichloroaniline, and chlorobenzenes for earthworms in soils differing in organic matter content is almost the same if it is based on concentrations in the soil pore water. These pore-water concentrations were calculated using adsorption coefficients. Perhaps for plants an indication of the bioavailability of chemicals could also be obtained by using adsorption coefficients (K_p).

In this study, therefore, the possible relation between phytotoxicity in soil and nutrient solution is investigated by recalculating soil EC50 values ($EC50_s$ in micrograms per gram) to pore-water concentrations ($EC50_w$ in milligrams per liter), using the following equation:

$$EC50_w = 1/[(K_p/0.3) + 1] \cdot (EC50_s/0.3) \quad (1)$$

The factor of 0.3 in this equation is based on the water content of the soil, which was considered to be about 30% of dry weight (in reality, it fluctuated between 25 and 30%).

Adsorption to organic matter increases with increasing lipophilicity of the compound. Several equations have been described in the literature relating adsorption to lipophilicity. These equations may be used to calculate K_p values. For this purpose the following equation is used (after [63]):

$$K_p = 0.5 \cdot f_{oc} \cdot K_{ow} \cdot fr_{nd} \quad (2)$$

where

K_p = adsorption coefficient (in ml/g)

f_{oc} = fraction organic carbon (Organic matter is considered to contain approximately 58% organic carbon. The organic matter content of the test soils (1.4 or 1.8%) therefore corresponds with a fraction organic carbon of 0.0081 or 0.0104, respectively.)

K_{ow} = *n*-octanol/water partition coefficient of the compound

fr_{nd} = fraction nondissociated.

Lipophilicity decreases when compounds dissociate [63]. Chloro(methyl)phenols may dissociate, depending on the pH of the soil and/or nutrient solution and the pK_a of the compound. High dissociation rates occur if $pH > pK_a + 1$ [63], in which case adsorption is also dependent on soil pH. To correct for this, Equation 2 should be extended with a factor fr_{nd} $(1/(1 + 10^{pH-pK_a}))$.

With the adsorption coefficient K_p, it is possible to transform concentrations in soil to concentrations in the soil pore water, using Equation 1.

For compounds with EC50 values in soil >1,000 μg/g and those with EC50 values exceeding water solubility, EC50 values in soil and nutrient solution cannot be compared. These compounds were left out, and so were the compounds for which no

Table 3. Relationship between the toxicity of phenols, chlorobenzenes, chloroanilines, and miscellaneous compounds for *Lactuca sativa* in soil solution (calculated 7-d EC50 values in log μmol/L) and in nutrient solution (measured 16- or 21-d EC50 values in log μmol/L)

Compounds	Regression line	r	n
Phenols	$y = 1.08x + 0.58$	0.95	17
Chloroanilines	$y = 1.16x + 0.15$	0.90	12
Chloro(nitro)benzenes	$y = 1.48x - 0.21$	0.86	13
Miscellaneous	$y = 1.30x + 0.27$	0.87	19

K_p value could be calculated due to the lack of a log K_{ow} value.

Calculated soil-pore-water EC50 values and experimentally determined nutrient-solution EC50 values were compared by regression analysis, and the results are shown in Table 3. As soil-pore-water concentrations after 14 d were almost identical to those after 7 d, only the latter are used in Table 3. For ethylenediamine and azinphos methyl, no 7-d EC50 value could be calculated; therefore 14-d EC50 values were used. From Table 3 it can be concluded that there is a good correlation between estimated EC50 values in the soil pore water and values determined in nutrient solution. It is, however, also obvious that estimated soil-pore-water EC50 values are not identical to the values determined in nutrient solution. This difference may be due to differences in exposure (static vs. semistatic) and test period (7 and 16 or 21 days, respectively), which resulted in higher EC50 values in soil pore water. Furthermore, it should be realized that quite a number of assumptions had to be made to estimate soil-pore-water concentrations. K_p was based on calculations (Eqns. 1 and 2). Comparison of literature data on chlorophenols [63], chlorobenzenes [64,65], and naphthalene and acenaphthene [65–67] showed that the calculated values may be lower as well as higher than experimentally derived values. The difference is generally no more than a factor of five. Another important assumption is that adsorption increases linearly with the soil concentration. Lagas [68] showed that in a soil comparable to the soil used in this study, this is not the case for the lower chlorinated phenols. Nevertheless, the correlation between EC50 values obtained in nutrient solution tests and the calculated EC50 values for pore water suggests that for *L. sativa* the concentration in the soil pore water also is an important factor in the phytotoxicity of organic chemicals.

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