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Ecological Effects of PESTICIDES on Non-Target Species

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David Pimentel, Department of Entomology and Limnology,
Cornell University, Ithaca, New York

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2,4-D

Mammals

The LD_{50} for the rat was 666 mg/kg; for the mouse, 375 mg/kg; for the rabbit, 800 mg/kg; for the dog, 100 mg/kg; and for the guinea pig, 1,000 mg/kg to 2,4-D when the mammals were fed the stated dosages orally (Spector, 1955). The LD_{50} of 2,4-D for mule deer was given at 400 to 800 mg/kg when fed orally in capsules (Tucker and Crabtree, 1970).

Corn plants grown in soil treated with 2,4-D as a pre-emergent treatment (1 to 3 lb/A) were more attractive (about 33 percent more were destroyed) to mice than plants grown in untreated soils (Raleigh and Patterson, 1948). Most of the plants at the time of injury were in the three-leaf stage and about 3 inches tall, but only the kernel of the dug plants was eaten.

Vegetation treated with 2,4-D (alkanolamine salt) in a 5-percent solution repelled cattle (Grigsby and Farwell, 1950).

2,4-D has been reported to repel some mammals (Richter, 1952 in Springer, 1957). For example, cottontail rabbits given a choice of feeding on 2,4-D-treated vegetation or untreated vegetation ate almost none of the treated vegetation.

Treating vegetation with herbicides may alter the plant species composition, and thus the suitability of the habitat for certain mammals. For example, spraying mountain rangeland in Colorado with 2,4-D resulted in several changes in the normal vegetational types and, in turn, in the mammals after one year: (1) the production of perennial forbs was reduced 83 percent, and grass production increased 37 percent after treatment; (2) the diet of pocket gophers changed from 82-percent forbs to 50-percent forbs, and changed from 18-percent grass to 50-percent grass; and (3) the pocket gopher population was reduced 87 percent (Keith, Hansen and Ward, 1959). The suggested reasons for the decline in number of gophers were a depletion in the amount of essential food plants and nitrate poisoning.

In an investigation of the effect of sagebrush control by 2,4-D on use of vegetation by cattle and wildlife in Colorado, Anderson (1960) reported few changes in animal use during the short period of one year. He reported a small decrease in deer use in some of the treated areas. Anderson recommended that to be able to evaluate fully the effect of sagebrush eradication on deer, sage grouse, rabbits, and other animals, the investigation be carried on for several years.

2,4-D and 2,4,5-T at 4, 8, 12, and 16 lb/A improved deer browse by killing off tops of the taller trees and stimulating regrowth at the bases (Krefting and Hansen, 1963). Both herbicides proved most effective at the 12-lb dosage, and 2,4-D was significantly better than 2,4,5-T. The deer showed no preference for either untreated or herbicide-stimulated branch growth.

Lundholm (1970) reported that about 40 percent of a reindeer herd of 600 died in April and May, 1970, when they fed on coniferous vegetation which had been treated on July 12, 1969, with a mixture of 2,4-D (2 parts) and 2,4,5-T (1 part) at a rate of about 2.5 lb/A. Also, 40 of the reindeer aborted their young. Analyses revealed that the coniferous leaves from the area during April and May contained 25 ppm of 2,4-D and 10 ppm of 2,4,5-T.

Birds

The LD_{50} for young mallards was $>>$1,000 (acid) mg/kg; for young mallards, $>>$2,025 (sodium salt) mg/kg; for young pheasants, 472 (acid) mg/kg; for young coturnix, 668 (acid) mg/kg; and for pigeons (*Columba livia*), 668 (acid) mg/kg to 2,4-D when the birds were given the stated dosages orally in a capsule (Tucker and Crabtree, 1970). The LC_{50} for mallards was $>$5,000 ppm; for pheasants, $>$5,000 ppm; for bobwhites, $>$5,000 ppm; and for coturnix, $>$5,000 ppm of 2,4-D (BEE and dimethylamine salt) in diets of 2-week-old birds when fed treated feed for 5 days followed by untreated feed for 3 days (Heath et al., 1970).

2,4-D influenced egg production in chickens exposed for 14 days to grass sprayed daily with 2,4-D (32-percent acid) at ¼ oz/gal of water and 2½ oz/gal (Dobson, 1954). The lower 2,4-D treatment led to a 22-percent reduction in egg yield and the higher dosage, to only an 8-percent reduction, but there was no change in the fertility or hatchability of the eggs, nor did the chickens lose any weight. 2,4-D was also found in one test to depress total reproduction of mallard ducks when fed daily at rates of 1,250 and 2,500 ppm, and in another test at the same dosages reproduction was about 80-percent suppressed (USDI, 1970a).

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Wild turkeys used the treated right-of-way areas (2,4-D and 2,4,5-T) (Bramble and Byrnes, 1958). The young turkeys were attracted to the openings to feed on various insects more abundant on the grassy right-of-way than within the wooded areas.

Fishes

See table 57 for the LC_{50} for various fish to 2,4-D.

Spot were able to survive a 48-hour exposure to 50 ppm of 2,4-D without any deleterious effect (Butler, 1963).

In laboratory experiments 2,4-D was not toxic to bluegill or largemouth bass at 1 ppm, and only slightly toxic at 100 ppm (King and Penfound, 1946). Then Hiltibran (1967) reported that bluegill, green sunfish, and smallmouth bass fry survived a concentration of 10 ppm of 2,4-D (ethylhexy ester) for 8, 4, and 5 days, respectively, in an experiment lasting 8 days.

In India 2,4-D applied at a rate of 2.5 percent in 100 gallons/A killed 5 percent of the tadpoles, 1.6 percent of the Rahu fish fry, and 3.2 percent of the katla fish fry (Sen, 1957). However, no mortality was observed in native fish in an east-coast estuary when 2,4-D was applied at a rate of 30 lb/A (Beaven, Rawls and Beckett, 1962). Rawls in later investigations (1965) found that 2,4-D acetamide applied to an estuary at 20 lb/A killed all the caged fish (mostly pumpkinseed) within 30 days. 2,4-D butyl ester (BE) or isooctyl ester (IOE) formulations caused little or no mortality to the fish, and these formulations were judged as safe for use against milfoil in marshes.

Mortality among bluegills ranged from 19 to 100 percent in ponds treated with 10 ppm of 2,4-D (Wallen, 1963). Spawning was delayed for 2 weeks in the ponds with 10 ppm; however, fry production appeared to be essentially the same at 10, 5, 1, 0.5, and 0.1 ppm of 2,4-D.

Bluegills were found to convert the herbicide 2,4-DB to 2,4-D (Gutenmann and Lisk, 1965).

Young silver salmon when exposed to a combination of 2,4-D and 2,4,5-T (about 10 percent of each chemical in the combined formulation) at concentrations of 50 ppm or more were observed to be "immediately distressed and would snap their jaws, dart about the aquarium, and leap out of the water before loss of equilibrium and death" (Holland et al., 1960).

The LC_{50} for bluegill to 2,4-D formulations is presented in table 58 (Hughes and Davis, 1963). The ester formulations appeared to be most toxic to the fish, probably due to more effective penetration. Hughes and Davis did not attempt to explain the wide variation in results obtained from the different batch lots of the same formulation.

A group of experimental ponds were treated with 2,4-D at concentrations of 0.1, 0.5, 1, 5, and 10 ppm (Cope, Wood and Wallen, 1970). About 19 percent of the bluegills died within 8 days with

TABLE 57. The LC_{50} for various fish to 2,4-D.

Formulation	Fish Species	Exposure Time (hr)	LC_{50} (ppm)	Source
Butyl Ester	Harlequin fish	24	1.0	Alabaster, 1969
Oleic-1,-propylene diamine	Bluegill	24	4.0	Davis and Hughes, 1963
Butyl Ester	Bluegill	24	4.9	"
Butyl Ester	Bluegill	24	10	"
	Rainbow trout	24	250	Alabaster, 1956
Amine	Rainbow trout	24	250	Alabaster, 1969
Ethylhexy Ester	Lake Emerald shiner	24	280	Swabey and Schenk, 1963
Ethylhexy Ester	Lake Emerald shiner	24	620	"
Sodium Salt	Harlequin fish	24	1,160	Alabaster, 1969
Isopropyl	Bluegill	48	0.8	FWPCA, 1968
Propylene Glycol Butyl Ether Ester	Rainbow trout	48	0.96	"
	Rainbow trout	48	1.1	Bohmout, 1967
Butyl Ester	Bluegill	48	1.3	FWPCA, 1968
Mixed Butyl and Isopropyl Esters	Bluegill	48	1.5	"
Butoxyethanol Ester	Bluegill	48	2.1	"
	Bluegill	48	3.7	Bohmont, 1967

the highest concentration. At 5 ppm and below mortality was negligible. Growth in weight was nearly 3 times that of the control fish in the pond treated with 10 ppm of 2,4-D. Growth at the other 2,4-D concentrations was also greater than in the control, but not as great as in the 10-ppm concentration. The most severe pathologic lesions were observed in fish at the highest concentrations, and this lasted for nearly 84 days. The pathologic effects involved the liver, vascular system, and brain.

TABLE 58. The LC_{50} for bluegill to 2,4-D formulations, including different batches of same formulation (Hughes and Davis, 1963).

Formulation	LC_{50} (Acid Equiv. ppm) 24 hr	48 hr
2,4-D		
Alkanolamine, ethanol and isopropanol series	900	840
Alkanolamine, ethanol and isopropanol series	588	530
Alkanolamine, ethanol and isopropanol series	450	435
Dimethylamine	542	458
Dimethylamine	500	416
Dimethylamine	390	353
Dimethylamine	273	273
Dimethylamine	220	220
Dimethylamine	166	166
Di-N,-V-dimethylcocoamine	1.5	1.5
2,4-D acid, with emulsifiers	8.0	8.0
Isooctyl ester	66.3	59.7
Isooctyl ester	36.0	36.0
Isooctyl ester	8.8	8.8
Propylene glycol butyl ether ester	2.1	2.1
Butoxyethanol ester	2.1	2.1
Butyl ester	1.3	1.3
Mixed butyl and isopropyl esters	1.7	1.7
Mixed butyl and isopropyl esters	1.6	1.5
Isopropyl ester	0.9	0.8
Ethyl ester	1.4	1.4

In laboratory experiments conducted by Mr. Jack Lowe, fish were exposed to 2,4-D and carbaryl (no dosage given) for 1 to 5 months (Butler, 1969). The exposed fish grew as well as the controls and had little mortality; however, careful examination revealed massive invasions of the central nervous system of the test fish by what appeared to be a microsporidian parasite. The author suggested that the pesticides lowered the natural resistance of the fish to parasite attack.

An investigation of the persistence of 2,4-D in fish revealed that 50 percent of the chemical was lost in <1 week (Macek, 1969).

Amphibians

At 0.5-percent solution 2,4-D was found to inhibit the development of frog (*Rana temporaria*) eggs (Lhoste and Roth, 1946).

The 24-hour LC_{50} for chorus frog tadpoles exposed to 2,4-D was 100 ppm (Sanders, 1970).

Molluscs

The exposure of eastern oysters to 2.0 ppm of 2,4-D acid for 96 hours had no effect on shell growth (Butler, 1963).

Two weeks after an estuary in Virginia was treated with 2,4-D at a rate of 10 lb/A for milfoil (Haven, 1963), there was a significant reduction in the numbers of a small mollusc (*Macoma baltica*), and the population remained low for some 3 months. Haven felt that the reduction of the mollusc population was due to the decay of the milfoil and associated anaerobic conditions, and not directly to the herbicide.

Field application of 2,4-D with dosages as high as 120 lb/A did not affect caged eastern oysters and clams in the treated areas (USDI, 1962). Beaven, Rawls and Beckett (1962) also found 2,4-D safe for eastern oysters and soft-shell clams when applied at a rate of 30 lb/A. However, Rawls (1965) found that 2,4-D acetamide applied to an estuary at 20 lb/A killed all the caged eastern oysters and soft-shell clams within 30 days. But Rawls did find that 2,4-D BE and IOE formulations were safe for these molluscs.

Arthropods, Annelids, and Other Invertebrates

The LC_{50} for 2,4-D tested against the fiddler crab (*Uca pugnax*) at different times and dosages was as follows: 5,000 ppm for 96 hours, 2,500 ppm for 10 days, and 1,000 ppm for 17 days (Sudak and Claff, 1960).

The LC_{50} for various arthropods to 2,4-D is found in table 59.

The minimum lethal concentrations (ppm) of 2,4-D which produced a kill of fish-food organisms exceeding 25 percent are the following: *Daphnia*, 0.2; *Eucypris*, 0.6; *Hyallella*, 0.6; *Palaemonetes*,

TABLE 59. The LC_{50} for various arthropods to 2,4-D.

Formulation	Arthropod Species		Exposure Time (hr)	LC_{50} (ppm)	Source
Butoxyethanol ester	Amphipod	(*Gammarus lacustris*)	24	1. 4	Sanders, 1969
Propylene glycol butyl ester	"	(*G. lacustris*)	24	2. 1	"
Ilsooctyl ester	"	(*G. lacustris*)	24	6. 8	"
Butyl ester	Stonefly	(*Pteronarcys californica*)	24	8. 5	Sanders and Cope, 1968
	"	(*P. californica*)	24	56	"
Dimethylamine	Amphipod	(*G. lacustris*	24	>100	Sanders, 1969
Butoxyethanol ester	Stonefly	(*P. californica*)	48	1, 800	FWPCA, 1968
Propylene glycol butyl ether ester	Amphipod	(*G. lacustris*)	48	1, 800	"
Propylene glycol butyl ether ester	Waterflea	(*Daphnia pulex*)	48	3, 200	"

0.8; *Amphiagrion*, 3.0; *Pachydiplax* and *Tramea*, 4.5; *Culex*, *Aedes*, and *Anopheles*, 3.5; *Chironomus*, 1.0; *Physa*, 5.5; and *Helisoma*, 7.5 (Zischkale, 1952).

Brown shrimp exposed to 2.0 ppm of 2,4-D for 48 hours showed a 10-percent mortality or paralysis (Butler, 1963). The median immobilization concentration of 2,4-D to *Daphnia magna* was found to be 100 ppm (Crosby and Tucker, 1966). This concentration for *D. magna* is about 500 times greater than that given for *Daphnia* above. There is no explanation given.

Two weeks after an estuary in Virginia was treated for milfoil control with 2,4-D at 10 lb/A, there was a significant reduction in the numbers of an amphipod (*Leptocherius plumulosus*) (Haven, 1963), and the population remained low for some 3 months (see comment under Molluscs).

No mortality was observed in native crabs exposed to 2,4-D applied at a rate of 30 lb/A (Beaven, Rawls and Beckett, 1962). Furthermore, field applications of 2,4-D as high as 120 lb/A did not kill caged blue crabs (USDI, 1962). Rawls (1965), however, found that 2,4-D acetamide applied to an estuary at 20 lb/A killed all the caged blue crabs within 30 days. But the 2,4-D BE or IOE formulations caused little or no mortality to the test animals, and these formulations were judged safe for use against milfoil in the marshes.

2,4-D applied at rates of 20 and 40 lb/A did not significantly influence the numbers or weight of bottom invertebrates (Hooper, 1958). However, Walker (1962) reported the following bottom organisms were reduced 50 percent or more one week after treatment with 2,4-D (1 to 4 ppm): mayfly nymphs, horsefly nymphs, common midges, phantom midges, biting midges, caddice fly larvae, water beetles, aquatic worms, and leeches (plus clams and snails). In another investigation no significant changes in numbers of burrowing mayflies (*Hemagenia*) were measured after the treatment of the reservoirs with 100 lb (1 ppb in water) per acre of 2,4-D (Smith and Isom, 1967). The conclusion from these investigations was that low concentrations of 2,4-D (20 to 100 lb/A, resulting in about 1 ppb of 2,4-D) have little effect upon bottom organisms.

Of mosquito larvae treated with 2,4-D at a rate of 100 ppm in water, about three-fifths fewer larvae as in the control reached the pupal state (Smith and Isom, 1967). This study added further evidence that 2,4-D is relatively non-toxic to some invertebrate species.

Water treated with 40 to 100 pounds per acre of 2,4-D for control of water milfoil did not appear to affect the aquatic fauna or water quality (Smith and Isom, 1967).

Jones and Connell (1954) calculated the LD_{50} of 2,4-D fed orally to honeybees at 104.5 μg/bee; however, Beran and Neururer (1955) reported an LD_{50} about $1/10$ this level, or 11.525 μg/bee.

Treating fields in New Zealand for ragwort control with 2,4-D at 3 lb/A caused a 22-percent mortality in honeybees working the treated field (Palmer-Jones, 1964). Dusting bees with 2,4-D, however, did not cause any mortality. Palmer-Jones raised the question whether the toxicity observed in the field was due to the 2,4-D dissolved in the nectar or to the production of a toxic metabolite secreted by the plant into the nectar.

2,4-D may also benefit bees, as reported by Beilmann (1950). The herbicide was used to restore bee pasture along the side of the road by destroying brush and other weeds, thus encouraging sweet clover to regain its dominance.

Fox (1964) reported that 2,4-D at 1 and 2 lb/A increased the wireworm (*Ctenicera aeripennis destructor*) damage to wheat. At 1 lb/A 31 percent of the wheat plants were killed, whereas in the untreated check only 5 percent were killed. The exact reason for the increased kill of wheat plants is not known, but one proposed reason was that 2,4-D delayed the growth of plants, thus making them more susceptible to wireworms.

Putnam (1949) reported that the number of grasshoppers (*Melanoplus mexicanus*) per square yard was about double in the 2,4-D (1 lb/A)-treated plots: 59 per sq. yd. in the 2,4-D-treated, compared with 30 per sq. yd. in the check. The indications were that 2,4-D hastened the development or increased the survival of the grasshoppers.

Dipping bean plants into 2,4-D at levels of 4.1 ppm increased aphid progeny production during a 10-day period from 139 to 764 per aphid (Maxwell and Harwood, 1960). In other experiments with a high dosage of 41.0 ppm of 2,4-D aphid production was less stimulated than at the lower dosage of 4.1 ppm. Some amino acids were at higher levels in the treated plants than in the untreated plants, and this probably improved the food resource for the aphids.

The longevity of aphid adults and the growth rate of grasshopper numphs appeared to be unaffected by the 2,4-D treatments (Maxwell and Harwood, 1960).

Adams (1960) reported that 2,4-D sprayed at a rate of ½ lb/A on coccinellid beetle larvae (*Coccinella transversoguttata*, *C. perplexa*, and *Hippodamia tredecimpunctata*), especially those in the late larval stages, killed between 70 and 75 percent of the animals. Also, the mean developmental time of the treated larvae increased significantly, in some cases from 16 to 27 days.

Aphids (primarily *Rhopalosiphum padi*) were more abundant on oats in fields treated with 2,4-D at ½ lb/A (Adams and Drew, 1965). Aphid outbreaks occurred, they suggested, because there were fewer coccinellid predaceous-beetles present in the treated areas, and the activity of the coccinellids present was depressed.

Rice stem-borer larvae grew almost 45 percent larger (35.1 mg on 2,4-D versus 24.4 mg for the control larvae) during the 30-day experimental period when rice plants on which the larvae fed were treated with 2,4-D (Ishii and Hirano, 1963). But when 2,4-D was added to sterilized rice stems fed to the rice stem-borer, larval growth was not improved. The explanation given by the authors was that 2,4-D increased the introgenous level in the growing rice plants, improving the plants as food for the larvae.

No mortality occurred in earthworms when they were immersed for 2 hours in concentrations of 0.1, 1.0, 10.0, and 100 ppm of 2,4-D, but at 1,000.0 ppm 100-percent mortality occurred (Martin and Wiggans, 1959).

2,4-D at normal dosages did not affect the numbers of wireworms, springtails, mites, and other micro-arthropods in soil (Van der Drift, 1963 and Rapoport and Cangioli, 1963).

Red clover plants resistant to the nematode *Ditylenchus dipsaci* lost this resistance when the plants were treated with 2,4-D (Webster and Lowe, 1966). Susceptible clover plants were made more attractive to nematodes after their treatment with 2,4-D. Red clover is not a normal host to the nematode *Aphelenchoides ritzemabosi*, but the nematode fed on the tissues treated with 2,4-D, significantly increasing the nematode's rate of reproduction. Webster and Lowe also found that 2,4-D greatly increased the reproduction of the nematode *A. ritzemabosi* in lucerne callus. They reported that soaking nematodes in 2,4-D solutions up to 5 ppm did not harm them, but that concentrations of 50 ppm did suppress their reproduction.

Spraying 2,4-D at a rate of 140 mg/sq. yd. onto nematode-susceptible and -resistant oats infested with *D. dipsaci* increased the number of nematodes per plant in both the resistant and susceptible cultivars (Webster, 1967). The number of nematodes was at least double in some treatments. Nematodes did not reproduce on the unsprayed resistant oat plants, whereas the nematodes associated with the 2,4-D treated plants produced a large number of eggs. The evidence suggested that in treated plants nematodes infesting the oats from the soil reproduced more than those inoculated directly into the oats.

Plants

Concord grapes were most sensitive to 2,4-D, and quantities as small as 0.0001 μg placed on a young leaf caused a malformation of from 4 to 6 leaves (Clore and Bruns, 1953). However, the exposure of phytoplankton to 1.0 ppm of 2,4-D for 4 hours did not cause a decrease in growth (Butler, 1963).

A significant increase in protein content of the grain was noted in wheat grown in 2,4-D treated plots (Helgeson, 1947). Treatments with 2,4-D also increased the amount of protein in wheat in direct relationship to the amount of 2,4-D used (Erickson, Seely and Klagas, 1948). At a dosage of 4.6 lb/A the protein level in wheat was 15.5 percent, whereas in the untreated control the protein level was only 10.9 percent.

Beans grown as a second crop on the previously 2,4-D-treated soil were observed to have a lower level of protein than the control (Anderson and Baker, 1950).

Nitrogen was higher in wheat grain treated with 2,4-D than in untreated grain (Pande, 1954). The increase in nitrogen was associated with increasing concentrations of 2,4-D (3.25 to 6.5 lb/A). It is interesting to note that the nitrogen level in wheat also increased when weeds were hand-pulled from the plots. Fults and Payne (1956) reported that treating bean plants with 2,4-D spray (1,000 ppm) caused a significant decrease in total free amino acids, in contrast to an increase in total amino acid in sugar beet and potato.

Willard (1950) reported that 2,4-D altered the palatability of some plant species to animals, such as livestock eating Canada thistle, velvet-leaf, jimson weeds, wild parsnip, sunflowers, round-leafed mallow, and other unpalatable weeds. The ragwort weed, highly toxic to cattle, had a marked increase in sugar content after treatment with 2,4-D. The high sugar content made the plants attractive to cattle, but they were still highly toxic. Normally, livestock and wildlife will not feed on ragwort unless forced to do so.

A sugar-beet field was treated with a "sublethal" dosage of 2,4-D by mistake (the farmer treated with 2,4-D-contaminated toxaphene), resulting in disfigured plants with a high level of nitrate (Stahler and Whitehead, 1950). The 2,4-D treatment increased the level of potassium nitrate in the beets from 0.22 percent by dry weight to 4.50 percent. A 1.5-percent level is toxic to cattle.

Sublethal concentrations of 2,4-D caused the level of potassium nitrate in Canada thistle and Russian pigweed to double (Berg and McElroy, 1953); in Canada thistle, 1.36 to 2.64 percent by dry weight, and in Russian pigweed, 2.45 to 4.38 percent. Sublethal spray applications of 2,4-D on both mustard and sugar beet plants resulted in increased levels of nitrates in the plants, but the increase was not much above 10 percent in most cases (Whitehead, Kersten and Jacobsen, 1956). Pigweed, lambsquarter (*Chenopodium*), and smartweed (*Polygonum*) were found to have extremely high levels of nitrate after treatment with 2,4-D (Olson and Whitehead, 1940 in Willard, 1950).

After the treatment of 9 species of weeds with sublethal concentrations (0.25 lb/A) of 2,4-D, potassium nitrate content declined from 6 to 44 percent in 5 species and increased from 12 to 47 percent in the other 4 species (Frank and Grigsby, 1957). In *Eupatorium maculatum* the increase was 47 percent. The nitrate levels in these species and several other species of plants were sufficiently high to cause nitrate poisoning in livestock if consumed in large enough quantities.

After buckwheat was sprayed with 50, 100, 500, and 1,000 ppm of 2,4-D the sugar values in the stems and leaves increased and then fell to a very low level by the eighth day after treatment (Wort, 1951). Total nitrogen and protein nitrogen in the stems and roots increased with both time and concentration after the herbicide application.

Black cherry brush was treated until wet with a concentration of 2,4-D at 2,000 ppm (Grigsby and Ball, 1952). By the 15th day after treatment, the hydrocyanic acid (HCN) present in the cherry leaves had been reduced by about 88 percent (control = 91.9 mg/100 g fresh wt.; 2,4-D treated = 11.3 mg/100 g).

Swanson and Shaw (1954) demonstrated that 2,4-D caused an initial decrease in the quantity of hydrocyanic acid in Sudan grass, but 4 days after treatment there was an increase in HCN over the controls. Results of their tests showed that the hydrocyanic acid content of Sudan grass was increased by 36 percent (control, HCN 36 mg/100 g fresh wt. versus 2,4-D 49 mg/100 g) in plots treated with 1 lb/A of 2,4-D. Note that the LD_{50} for sheep of HCN as a free glucoside is about 4.5 mg/kg body weight (Coop and Blakley, 1950).

Fertig (1953) reported that nitrate content of lambsquarter and pigweed may increase as much as 5.5 percent (dry weight). This would mean that 20 to 25 pounds of fresh green material would be toxic to a 500-pound animal.

Plants receiving high levels of nitrogen were more susceptible to 2,4-D than bean plants on low-N (Freiburg and Clark, 1951). 2,4-D also changed the absorbing capacity of bean-plant roots, as indicated by the failure of the treated plants to increase their content of total nitrogen, including nitrates, after exposure of 2,4-D. The treated bean plants showed a decrease in the percentage of protein and nitrogen in the leaves, but an increase in the stems and roots.

2,4-D applied to irrigation water at rates of 11 ppm caused tomato and cotton plants to grow more vigorously, but injured tokay and Concord grapes (Oborn, 1954).

Spraying 2,4-D and 2,4,5-T herbicides (2 quarts 2,4-D and 1 quart 2,4,5-T per 100 gallons of water) at a height of 4 to 6 feet along a roadway caused extensive damage to white, scarlet, and black oaks, plus other trees and shrubs (Niering, 1959).

Blaisdell and Mueggler (1956) reported that when 2,4-D was used at 1.5 to 2 lb/A on 15 shrubs and trees in the treated area, only serviceberry, threetip sagebrush, and silver sagebrush suffered moderate to heavy mortality. Aerial portions of snowbrush, downy rabbitbrush, aspen, chokecherry, willows (*Salix* sp.), and snowberry were affected, but most of these species sprouted profusely later. Bitterbrush, a valuable forage species, was unharmed or only slightly damaged. These authors point out that because of the differences in response of various associated forbs, shrubs, and trees, vegetational composition should always be considered when planning brush control.

Microorganisms

Worth and McCabe (1948) reported that when 2,4-D solutions of 1 and 2 percent were used in the medium, the herbicide inhibited the growth of the aerobic organisms, but had little effect on the facultative anaerobes. In some instances, the growth of the anaerobes was actually stimulated.

The herbicide 2,4-D did not inhibit ammonifying bacteria at concentrations of 0.25 percent and below (Jones, 1956). This was well below the rate ordinarily added to soil.

2,4-D at concentrations below 1,000 mg/l was observed to have little effect on bacterial growth of *Bacterium lactis aerogenes* (Dean and Law, 1964).

Anderson and Baker (1950) reported some inhibition of microorganisms, especially the gram-positive organisms, in the soil with 2,4-D at normal application rates; however, this inhibition of growth was quite transitory. Beans grown as a second crop on the treated soil had a lower level of protein than the control.

At normal field applications of 2,4-D (1 to 4 lb/A) the herbicide had little effect on soil microorganisms (Kratochvil, 1950; Hoover and Colmer, 1953; Fletcher, 1960; and Bounds and Colmer, 1964).

At a dosage of 50 ppm of 2,4-D nitrification in the soil was completely inhibited (Slepecky and Beck, 1950). After the treatment of the soil with 2,4-D an actinomycete was reported as dominating the soil flora (Warren, Graham and Gale, 1951). The actinomycete had rather strong anti-fungal properties, and thus had an inhibiting effect upon soil fungi.

2,4-D applied in 0.1-, 0.5-, and 1-percent solutions favored the increase of soil microorganisms (Il'in, 1962). The 1-percent 2,4-D caused protozoa to cease their activity in 1 to 2 seconds and form cysts. The increase in the number of soil microorganisms may be due to the inhibition of protozoa in the soil. Rapoport and Cangioli (1963) supported this idea, reporting that using sodium salt of 2,4-D inhibited soil protozoa and resulted in an increase in the number of bacteria. 2,4-D stimulated the growth of saprophytic microorganisms in water (Petruk, 1964).

At 10 and 50 ppm of 2,4-D, *Aspergillus niger* proliferation was significantly limited (Arnold, Santelmann and Lynd, 1966). *A. niger* degraded 2,4-D faster than it did picloram, which had little effect upon the fungus.

Biological Concentration

The eastern oyster concentrated 0.1 ppm of butoxy-ethanol ester of 2,4-D in water to a level of 18.0 in itself during 7 days, as measured by 2,4-D acid (Butler, 1965). When a sample of these oysters was placed in clean water for 7 days, the 2,4-D disappeared from the bodies of the oysters.

Esters of 2,4-D accumulated in sunfish after exposure to sublethal concentrations in both laboratory and field tests (Cope, 1965b), and the fish sampled from a reservoir with 1 ppb showed an uptake of 2,4-D to a maximum of 150 ppb (Smith and Isom, 1967).

Within an hour after being treated with 2,4-D at a rate of 100 lb/A, the concentration of 2,4-D in reservoir water was about 1 ppb (Smith and Isom, 1967). Mussels (primarily *Elliptio crassidens*) exposed to the water for 96 hours concentrated the 2,4-D: 2 samples of mussels had an average of 380 ppb and 700 ppb of 2,4-D in their tissues. Asiatic clams concentrated 2,4-D to <140 ppb.

Resistance

Abel (1954) has noted that increasing doses of 2,4-D were required to control creeping thistle, which suggests the development of resistance in this weed.

There was evidence that strains of broad-leaved plants with a relatively high inherited tolerance for 2,4-D have been selected chemically in the spraying process in sugar-cane fields since 1945 (Hanson, 1956).

Persistence

The evidence suggests that under normal use 2,4-D persists in soil for about a month. In moist-loam soil 2,4-D applied at a rate of ½ to 3 lb/A persisted for 1 to 4 weeks with little or no leaching, under summertime conditions in a temperate climate (Klingman, 1961). Sheets and Harris (1965) reported also that 2,4-D at normal recommended dosages persisted for about 1 month in the soil.

2,4-D and 2,4,5-T was applied as a 1:1 mixture as low volatile esters in diesel oil at rates of 2 lb/A (Norris, 1967). From a maximum of 70 ppb, the residue dropped rapidly to less than 0.5 ppb a few days after spraying. Four gallons of diesel oil per acre had little or no effect on the decomposition of 2,4-D in forest litter; in other tests, DDT applied at 1 lb/A appeared to stimulate the breakdown of 2,4-D (Norris and Greiner, 1967).

2,4-D appears to degrade rather rapidly in water. For example, the concentration dropped from 1,000 ppm of application rate to 10 ppb within 30 days (House et al., 1967). However, significant concentrations of 2,4-D (58.8 ppm) were recorded and isolated from sediment samples removed from a reservoir some 10 months after treatment (Smith and Isom, 1967).

From 1 to 2 percent of the 2,4-D applied at a rate of 689 ppb to 967 ppb to water, remained for 31 days after treatment (Averitt, 1967). The most rapid decline, occurring about 4 days after application in the 2 lagoons treated, was not due to heavy rainfall. The author could give no explanation for this rapid loss.

2,4-D applied at 4 lb/A persisted in soil for 4 to 18 weeks (Hernandez and Warren, 1950).

At 10 ppm 2,4-D was found to persist in ponds in Oklahoma for 6 weeks, although in bluegill fish none was detected after 4 days (Cope, Wood and Wallen, 1970).

DALAPON

Mammals

The LD_{50} for the rat was 7,570 to 9,330 mg/kg; for the female mouse, >4,600 mg/kg; for the female rabbit, 3,860 mg/kg; and for the female guinea pig, 3,860 mg/kg to dalapon when the mammals were fed the stated dosages orally (WSA, 1967).

Birds

Several species of birds survived when their diet contained as much as 5,000 ppm of various herbicides. Dalapon was found to depress reproduction in mallard ducks when fed at levels of less than 25 percent of those which produced mortality (USDI, 1962).

The LC_{50} for mallards was >5,000 ppm; for pheasants, >5,000 ppm; and for coturnix, >5,000 ppm of dalapon in diets of 2-week-old birds when fed treated feed for 5 days followed by untreated feed for 3 days (Heath et al., 1970).

Fishes

When Lake Emerald shiners were exposed for 3 days to 3,000 ppm of dalapon, no adverse effects were observed (Springer, 1957). See table 60 for LC_{50} for various fish to dalapon. There appears to be some discrepancy in the toxicity of dalapon to bluegills.

Longnose killifish exposed to 1.0 ppm of dalapon (sodium salt) for 48 hours exhibited no noticeable effects (Butler, 1963).

Hiltibran (1967) reported that bluegill, green sunfish, lake chub-sucker and smallmouth bass fry survived a concentration of 50 ppm of dalapon for 8 days or the termination of the experiment.

Molluscs

The exposure of the eastern oyster to 1.0 ppm of dalapon (sodium salt) for 96 hours had no noticeable effect on shell growth (Butler, 1963).

Sodium pentachlorophenate applied to a creek at the rate of 9.5 ppm was reported to kill all catfish (*Ictalurus*), guppies, and eels (Springer, 1957).

Arthropods

Crayfish exposed to 9.5 ppm of sodium pentachlorophenate in creek water were unharmed (Springer, 1957).

2,4,5-T

Mammals

The LD_{50} for the rat was 300 mg/kg and for the dog, 100 mg/kg to 2,4,5-T when the mammals were fed the stated dosages orally (Spector, 1955).

Rowe and Hymas (1954) presented data indicating that the acute oral LD_{50} of 2,4,5-T to various species of mammals was about 500 mg/kg.

Birds

The LC_{50} for mallards was >5,000 ppm; for pheasants, 1,250 to 2,500 ppm; and for coturnix, >5,000 ppm of 2,4,5-T in diets of 2-week-old birds when fed treated feed for 5 days followed by untreated feed for 3 days (Heath et al., 1970).

The use of 2,4,5-T and 2,4-D for brush control under power lines improved the environment for ruffed grouse, as measured by an increase in grouse numbers (Bramble and Byrnes, 1958). The grouse were found on the edges within 150 to 200 feet of the right-of-way, rather than on the right-of-way itself. This emphasized the importance of the right-of-way as a creator of edge effects. Wild turkeys also made effective use of the right-of-way treated areas. The young turkeys were attracted to the cleared area for feeding on various insects, which were more abundant on the grassy right-of-way than within the wooded areas.

Sublethal concentrations of 2,4,5-T may have significant effects upon biological activities in birds. Chickens were exposed for 14 days to grass sprayed daily with 2,4,5-T (15-percent active agent) at ½ oz/gal of water and 2½ oz/gal (Dobson, 1954). The lower dosage led to a 9-percent reduction in egg yield, and the higher dosage to an 18-percent reduction, but there was no change in the fertility or hatchability of the eggs. The exposed chickens also lost some weight.

Fishes

See table 76 for the LC_{50} for various fish to 2,4,5-T.

When young silver salmon were exposed to a combination of 2,4,5-T and 2,4-D (about 10 percent of each chemical in the formulation) at concentrations of 50 ppm or more they were "immediately distressed and would snap their jaws, dart about the aquarium, and leap out of the water before loss of equilibrium and death" (Holland et al., 1960).

Mullet exposed to 50 ppm of 2,4,5-T for 48 hours exhibited no noticeable effects (Butler, 1963).

The 24-hour LC_{50} of bluegills to various 2,4,5-T formulations are presented in table 77 (Hughes and Davis, 1963). The ester formulations appeared to be most toxic to the fish probably due to more effective penetration. No attempt was made by Hughes and Davis to explain the wide

TABLE 76. The LC_{50} for various fish to 2,4,5-T.

Formulation	Fish Species	Exposure Time (hr)	LC_{50} (ppm)	Source
Butyl ester	Harlequin fish	24	1.0	Alabaster, 1969
Isopropyl ester	Bluegill	24	1.8	Davis and Hughes, 1963
Oleic-1,3-propylene diamine	Bluegill	24	2.9	"
Acid	Rainbow trout	24	12	Alabaster, 1956
Triethylamine	Bluegill	24	53.7	Davis and Hughes, 1963
Acid	Bluegill	48	0.50	Bohmont, 1967
Propylene glycol butyl ether ester	Bluegill	48	0.56	FWPCA, 1968
Acid	Rainbow trout	48	1.3	Bohmont, 1967
Isopropyl ester	Bluegill	48	1.7	FWPCA, 1968
Isooctyl ester	Bluegill	48	16.7	"

variation in results obtained from the different batch lots of the same formulation.

Hiltibran (1967) reported that bluegill and green sunfish fry survived a concentration of 10 ppm for 8 days or the termination of the experiment.

TABLE 77. The 24-hour LC_{50} of bluegills to 2,4,5-T formulations (Hughes and Davis, 1963).

2,4,5-T	LC_{50} (ppm)
Dimethylamine	144
Isooctyl ester [1]	31
Isooctyl ester [1]	28
Isooctyl ester [1]	10.4
Propylene glycol butyl ethyl ester	17
Butoxyethanol ester	1.4

[1] Different batches of same formulation.

Molluscs

The exposure of oysters to 2.0 ppm of 2,4,5-T acid for 96 hours had no effect on shell growth (Butler, 1963).

Arthropods

The minimum lethal dosages (ppm) which produced a kill exceeding 25 percent with 2,4,5-T are listed for the following fish-food organisms: *Daphnia*, 1.5; *Eucypris*, 0.5; *Hyallella*, 0.7; *Palaemonetea*, 1.2; *Amphiagrion*, 7.5; *Pachydiplax* and *Tramea*, 8.0; and *Chironomus*, 6.0 (Zischkale, 1952).

The exposure of brown shrimp to 1.0 ppm of 2,4,5-T for 48 hours had no deleterious effects (Butler, 1963).

Plants

Fifteen days after black-cherry brush had been treated until wet with a 2,4,5-T concentration of 2,000 ppm, Grigsby and Ball (1952) reported that the hydrocyanic acid (HCN) content was reduced 85 percent (control=91.9 mg/100 g fresh wt; 2,4,5-T=10.8 mg/100 g).

Swanson and Shaw (1954) demonstrated that the hydrocyanic acid content of Sudan grass was increased by 69 percent (control, HCN 36 mg/100 g fresh wt. versus 2,4,5-T, 61 mg/100 g) in plots treated with 1 lb/A of 2,4,5-T.

When 9 species of weeds were treated with sublethal dosages (0.25 lb/A) of 2,4,5-T, the nitrate content of the plants decreased from 5 to 32 percent in 4 species and increased from 3 to 36 percent in 5 other species (Frank and Grigsby, 1957). The 36-percent increase (control, 9.8 mg/g dry wt. versus 2,4,5-T, 13.6 mg/g) in potassium nitrate occurred in *Impatiens biflora*.

The exposure of phytoplankton to 1.0 ppm of 2,4,5-T for 4 hours caused no decrease in productivity (Butler, 1963).

Microorganisms

Magee and Colmer (1955) reported that 2,4,5-T at 1,500 to 2,000 ppm produced an inhibition of respiration to *Azotobacter* sp. Bounds and Colmer (1964), however, found that 2,4,5-T did not affect *Streptomyces* at 2 and 50 lb/A.

Persistence

2,4,5-T applied at 5 ppm to soil persisted for 166 to >190 days (DeRose and Newman, 1947).

In moist-loam soil 2,4,5-T applied at a rate of ½ to 3 lb/A persisted for 2 to 5 weeks with little or no leaching, under summertime conditions in a temperate climate (Klingman, 1961). Sheets and Harris (1965), however, reported that 2,4,5-T generally persisted for about 3 months under moist soil conditions.

TAR DISTILLATE

Birds

Tar distillate had a significant effect when chickens were exposed daily for 14 days to grass sprayed daily with tar distillate winter wash (30 percent cresol and phenol as the active agents) at ½ pt and 2 pt made up to 1 gal of water (Dobson, 1954). The low tar-distillate treatment led to a 17-percent reduction in egg yield, and the higher dosage led to a 46-percent reduction, but there was no reduction in egg fertility or hatchability. The chickens did not lose weight after the exposure.

APPENDICES

Appendix A—Abbreviations Used

ppm, parts per million (parts in 10^6 parts) is the number of parts of toxicant per million parts of the substance in question (not necessarily in solution); these may include residues in soil, water, or whole animals.

ppb, parts per billion (parts in 10^9 parts), is the number of parts of toxicant per billion parts of the substance in question.

mg/kg, milligrams per kilogram, is used to designate the amount of toxicant required per kilogram of body weight of test organism to produce a designated effect, usually the amount necessary to kill 50 percent of the test animals.

μg, microgram, 1/1,000,000 of gram.

ng, nanogram, 1/1,000,000,000 of a gram.

LD_{50}, median lethal dose, is the milligrams of toxicant per kilogram of body weight lethal to 50 percent of the test animals to which it is administered under the conditions of the experiment.

LC_{50}, median lethal concentration, is the concentration (ppm or ppb) of toxicant in the environment (usually water) which kills 50 percent of the test organisms exposed.

EC_{50}, median effective concentration, is the concentration (ppm or ppb) of toxicant in the environment (usually water) which produces a designated effect to 50 percent of the test organisms exposed.