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ENDOSULFAN--
ENVIRONMENTAL BEHAVIOUR, FATE AND EFFECTS

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1.0 PHYSICAL AND CHEMICAL PROPERTIES

1.1 INTRODUCTION

Endosulfan is a broad spectrum, organochlorine pesticide used to control a wide range of insect and other invertebrate pests, including: aphids, thrips, beetles, foliar feeding larvae, mites, borers, cutworms, bollworms, bugs, termites, tsetse flies, whiteflies, leaf hoppers and slugs in citrus and small fruits, vegetables, forage, oil and fiber crops, grains, tobacco, coffee, tea, forest and ornamentals. Products containing endosulfan operate typically as non-systemic contact and stomach insecticides (Environment Canada 1990).

Common names include thiodan and benzoepin, but endosulfan is the name approved by the British Standards Institute (BSI), the International Standards Organization (ISO) and the American National Standards Institute (ANSI). Endosulfan is marketed under the trade names Thiodan, Endosulfan 400, Wilson's Endosulfan, Cyclodan, Beosit, Malix, Thimul and Thifor. The accepted IUPAC chemical name is 1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-en-2,3-ylene dimethyl sulphite (British Crop Protection Council 1979). Alternate names include: 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzo[e]dioxathiepin 3-oxide; 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin 3-oxide; 1,4,5,6,7,7-hexachloro-5-norbornene-2,3-dimethanol cyclic sulfite; and 1,2,3,4,7,7-hexachlorobicyclo-2,2,1- hepten-5,6-bisoxymethylene sulfite (Environment Canada 1990; British Crop Protection Council 1979).

Endosulfan was developed in Germany by Hoechst AG and licensed to be sold in the U.S. in 1956 by FMC Corporation (British Crop Protection Council 1979; Thompson 1989). It is currently sold in Canada under the trade names: Thiodan (Hoechst Canada Inc.), Endosulfan 400 (Pfizer C&G Inc.) and Wilson's Endosulfan (Wilson Laboratories Ltd.). It is available in the form of granules, dusts, wettable powders and emulsifiable concentrates (Environment Canada 1990).

1.2 PROPERTIES

Endosulfan is non-polar, reflected in its low solubility in water and moderate solubility in most organic solvents (octanol/water partition coefficient (K_{ow}) of 3.6). It is produced by reacting

thionyl chloride with the hydrolysed Diels-Alder adduct of hexachlorocyclopentadiene and (2)-but-2-enylene diacetate. The technical grade is a brownish crystalline solid (melting point of 70-100 °C; vapour pressure of 1 x 10^{-5} mm Hg at 25 °C), while pure endosulfan is a colourless, non-flammable, crystalline compound (vapor pressure of 9 x 10^{-3} mm Hg at 80 °C) having a faint smell of sulfur dioxide and hexachloropentadiene (Agriculture Canada 1982; British Crop Protection Council 1979). Endosulfan is corrosive to iron (Environment Canada 1990), which catalyzes its degradation, and is sensitive to both bases and acids (Agriculture Canada 1982).

Technical endosulfan is a mixture of two stereoisomers. α-endosulfan (endosulfan I) (C.A. Registry Number 959-98-8) and ß-endosulphan (endosulfan II) (C.A. Registry Number 33213-65-9). The α-isomer makes up 70% of the technical grade product and ß-endosulfan the remainder. Respective melting points of the two isomers are 108-110 °C and 208-210 °C (British Crop Protection Council 1979). Water solubility and octanol/water partition coefficients vary according to the specific isomer. Water solubilities at 22 °C and pH 7.2 for the α- and ß-isomers and the sulphate degradation product are 0.15, 0.06 and 0.22 $mg \cdot L^{-1}$ respectively. Solubility increases slightly at lower pH. The corresponding octanol/water partition coefficients are 3.55, 3.62 and 3.66 (Environment Canada 1987).

1.3 DEGRADATION PRODUCTS

The technical grade of endosulfan is relatively stable to sunlight and low pH and is compatible with non-alkaline pesticides, but is subject to slow hydrolysis to the alcohol form (diol) and sulphur dioxide, particularly at elevated pH (see Section 3.2.4) (British Crop Protection Council 1979). Both isomers of endosulfan can be oxidized to form endosulfan sulfate, a form which can be toxic to fish (Fox and Matthiessen 1982). A 1979 review listed the degradation products as: endosulfan sulphate, endosulfan alcohol (diol), endosulfan ether, endosulfan α-hydroxyether and endosulfan lactone (Gupta and Gupta 1979).

2.0 PRODUCT USE IN CANADA

Endosulfan is used in Canada typically for the control of wood borers and other insects on fruit and ornamental trees and shrubs, but can also be used to control insects on alfalfa, clover, corn, melons, potatoes, sunflowers, strawberries and tobacco (Environment Canada 1987). No products

containing endosulfan are restricted in Canada, and 11 products are registered for domestic (home) and commercial (industrial and agricultural) use. The compound is usually applied by aerial or ground spray techniques (Environment Canada 1987), at recommended rates of 0.25-0.1 $g \cdot L^{-1}$ of water or 0.22-4.4 kg/ha (Environment Canada 1990).

Use patterns in Atlantic Canada are not well known. A retail inventory of agricultural pesticides on Prince Edward Island revealed that 143 kg (active ingredient) of chlorinated insecticides including chlordane, endosulfan, and methoxychlor were used in the province in 1986, compared with sales in 1982 of 2,320 kg (Environment Canada 1988a). Although exact figures are not available, it has been estimated that 50 - 60% of the 30,000 ha of potato fields in Prince Edward Island are treated annually with Thiodan (an endosulfan formulation) and that its usage is increasing (B. Craig, pers. comm.).

A similar retail survey for Nova Scotia (Environment Canada 1988b) of the same pesticide group indicated total chlorinated insecticide sales of 658 kg (active ingredient) in 1986 compared with 2,700 kg in 1982. A more complete picture is available for New Brunswick, where sales of endosulfan increased from 285.6 kg (active ingredient) in 1986 to 2,274 kg in 1988, and the amount of product sold from 5,410 L in 1988 to 5,838 L in 1992 (K. Stapleton, pers. comm.). Endosulfan use in New Brunswick is summarized in Table 2-1. Endosulfan is not used in Newfoundland in measurable amounts. In general, there is insufficient information from these studies to allow changes in amounts used or use patterns to be determined.

3.0 ENVIRONMENTAL FATE

3.1 FATE OF ENDOSULFAN IN WATER

Once it has entered the aquatic environment, endosulfan is subjected to a range of physical/chemical and biological processes including: hydrolysis, photolysis, volatilization, sorption and bioaccumulation, which contribute, all or in part, to its degradation and subsequent fate (Environment Canada 1987). The stability of endosulfan in aqueous environments is greatly influenced by the presence of the cyclic sulfite ester group, and hydrolysis to the alcohol form seems to be the dominant chemical and biological reaction (Brooks 1974b). Endosulfandiol was identified as a degradation product in seawater, and hydroxide-catalysed hydrolysis has been indicated to be a major pathway for endosulfan degradation in marine systems (Cotham and

Bidleman 1989).

Laboratory and field studies have shown that a single addition of endosulfan to water has a relatively short residence time. For example, Eichelberger and Lichtenberg (1971) found that 95% of an initial concentration of 10 $\mu g \cdot L^{-1}$ endosulfan in raw river water at room temperature had dissipated after two weeks. Ferrando *et al.* (1992a,b) calculated a half-life for 500 $\mu g \cdot L^{-1}$ technical endosulfan in lake water to be 50.3 hours, and Greve and Wit (1971) estimated the half-life of endosulfan added to a pond to be two days. Longer half-lives were found for α- and ß-endosulfan in non-sterile seawater (4.9 and 2.2 days respectively, pH 8.0), and in a seawater-sediment system (22 and 8.3 days respectively at pH 7.3 - 7.7) (Cotham and Bidleman 1989). Nonetheless, long-term monitoring studies have shown that endosulfan persists in water from one season to the next, probably as a result of runoff from agricultural fields (Frank and Logan 1988).

Although a single dosage of endosulfan may dissipate relatively rapidly, the initial concentration may be acutely toxic. Wan (1989) determined that water in a ditch beside an agricultural field sprayed with endosulfan contained 1,530 $\mu g \cdot L^{-1}$ endosulfan 30 minutes after application. This represents 1,275 times the average 96-h LC50 for rainbow trout (see Section 6.2.2).

3.2 FATE OF ENDOSULFAN IN SOILS AND SEDIMENTS

3.2.1 Persistence and Distribution

Endosulfan readily binds to soils and to a wide variety of materials including riverine silt (Greve and Wit 1971), and accumulates in elevated concentrations in sediments. Wan (1989) indicated that because endosulfan and its transformation products had soil half-lives of 3 - 6 months or more, the potential for bioaccumulation and sub-lethal impacts on organisms probably exists.

Miles and Harris (1971) measured residues of endosulfan in a drainage ditch which collected runoff from 600 ha of agricultural land in Ontario and determined that concentrations in mud (<1 to 62 $\mu g \cdot kg^{-1}$) were 330 times greater than those in the ditch water (<0.0002 to 0.187 $\mu g \cdot kg^{-1}$). Residues in suspended particulates in a tidal creek reached concentrations of up to 280 $\mu g \cdot kg^{-1}$ following a runoff event, relative to 1.25 $\mu g \cdot L^{-1}$ in the water column, a ratio of 224:1

(Chandler and Scott 1991). Chandler and Scott (1991) also measured endosulfan concentrations in the sediments over a six-week period, during which there were weekly rainfall events, showing average sediment levels of 231 $\pm$ 52 $\mu g \cdot kg^{-1}$. Wan (1989) reported that ditch sediments adjacent to agricultural lands were consistently contaminated with endosulfan at concentrations ranging from 2 to 150 $\mu g \cdot kg^{-1}$ (mean 18.8 $\mu g \cdot kg^{-1}$).

Dissipation of endosulfan residues from soil has been evaluated in a limited number of studies. Rao and Murty (1980b) measured a concentration of 19,700 $\mu g \cdot kg^{-1}$ in soil immediately following an application of 0.23 kg/ha endosulfan to a cotton crop, and 350 $\mu g \cdot kg^{-1}$ after 60 days, a decline of 98%. Endosulfan sulfate was found to be the primary endosulfan metabolite. A slower disappearance rate was established by Wan (1989), who measured an endosulfan concentration of 1,712 $\mu g \cdot kg^{-1}$ in the top 5 cm of soil immediately after an application of 0.568 kg/ha endosulfan, and 73 $\mu g \cdot kg^{-1}$ after 140 days, a decline of 96%. There is evidence, however, of endosulfan persisting in soil from one year to the next. Frank *et al.* (1982) reported year-round losses due to storm runoff and internal soil drainage in 11 agricultural watersheds, while Wan (1989) observed pre-spray soil residues of 219 $\mu g \cdot kg^{-1}$ in farm soil.

Endosulfan isomers show different rates of dissipation from the soil. After technical endosulfan was incorporated into the top 15 cm of soil at a simulated application rate of 6.7 kg/ha, 50% of the α-endosulfan had disappeared after 60 days, versus 800 days for ß-endosulfan (Stewart and Cairns 1974). As in the Rao and Murty (1980) study, the principal degradation product was endosulfan sulfate, which showed a coincident increase in amount equivalent to decreases in endosulfan isomers. The relatively rapid degradation of endosulfan reported in Rao and Murty (1980) may be partly explained by the higher ambient air temperature compared to the other studies.

The bulk of endosulfan residues in soil in field situations is consistently bound in the surface layers (Stewart and Cairns 1974; Rao and Murty 1980b) (see also Section 3.2.2). Stewart and Cairns (1974) found 90% of the endosulfan residues in the 0 - 15 cm horizon, 9% at a depth of 15 - 30 cm, and 1% in the 30 - 45 cm section. Similarly, Rao and Murty (1980) determined that 95% of the endosulfan and sulfate residues 100 days after application were in the top 7.6 cm, while only traces of endosulfan isomers were found at a depth of 7.6 - 10.2 cm. Given this distribution of endosulfan, it appears that runoff, rather than leaching, would be the major factor in endosulfan movement.

The importance of runoff has been estimated in several other studies. Waucope (1978) estimated runoff losses of 1% of the amount applied for water insoluble pesticides like endosulfan which are applied as emulsions. During a catastrophic rainfall, involving prolonged, intense precipitation within three days of a pesticide application, losses greater than 2% could occur (Waucope (1978). Chandler and Scott (1991) estimated that up to 5% of the applied pesticide could run off fields as solubilized residues, undissolved particulates, and bound residues on eroded soils and plant debris.

Frank *et al.* (1982) examined the mechanisms of pesticide loss from agricultural lands to streams on a year-round basis and determined that for endosulfan, storm runoff (including snowmelt) was the most important mechanism, accounting for 81% of the loss. Other, lesser mechanisms included a 16% loss from spills, drift and direct application, and a minimal 3% loss from internal soil drainage. Losses were highest during spring runoff since the greatest volumes of water and soil were moved from the land to watercourses at that time. In a study in British Columbia, Wan (1989) ascribed elevated concentrations of endosulfan in ditch sediments during the wet season, to surface runoff. In another field study, Epstein and Grant (1968) found that the amount of endosulfan in runoff from potato fields in Presque Isle, Maine, from July to October 1966, totalled 3.69 g/ha or about 0.35% of the amount applied.

3.2.2 Leaching and Adsorption

The properties of endosulfan make it unlikely to leach appreciably from the soil, and a low tendency to leach has been confirmed in various studies. Based upon endosulfan's k_{oc} of 2,040, a hydrolysis half-life of 14 days, and a soil half-life of 120 days, Gustafson's (1989) nomogram for estimating chemical leachability indicates that endosulfan is an improbable leacher and is unlikely to pose a significant threat to ground water, except where back siphoning has occurred or where there may have been a spill adjacent to a well casing. In a laboratory study, El Beit *et al.* (1981b) found that neither α- nor ß-endosulfan applied as a mixture (100 $\mu g \cdot g^{-1}$) to Gezira soil leached through 17 cm soil columns, at temperatures up to 45 °C. Similarly, no leaching below a 17 cm depth was noted for either endosulfan isomer when applied as a mixture (43.2 $\mu g \cdot g^{-1}$) to a sandy clay loam and eluted every 10 days over a 300-day period (El Beit *et al.* 1981a). Approximately 70% of the α-endosulfan was found at 0-4 cm as opposed to less than 10% at 12-16 cm. Similarly, almost 65% of the ß-endosulfan occurred in the upper 4 cm, while none was found in the 12-16 cm layer (El Beit *et al.* 1981a). Most of the endosulfan thus resides in the

top layer of soil and there is little downward movement. The presence of endosulfan in the top layer of soils may increase its susceptibility to transport by runoff.

The content of organic matter and clay has been shown to affect adsorption and leaching of endosulfan in the soil. El Beit *et al.* (1981a) suggested that soils which were either rich in organic matter or heavy clay would probably adsorb higher amounts of endosulfan (the sandy clay loam in the above tests contained only 2.6% organic matter). Support for this suggestion came from an earlier study by Bowman *et al.* (1965) which compared recoveries of α- and ß-endosulfan from soils having similar compositions of clay, silt and sand, but differing in organic matter content. Following an eight-day incubation period at 45°C, recoveries increased as the content of organic matter rose (Table 3-1). Thus, while only 9% of the α-endosulfan was recovered from soil containing less than 1% organic matter, 87% was retained by a soil with 19% organic matter. A similar pattern was established for ß-endosulfan, except that the recovery rates were higher, ranging from 35 to 100% as the organic content increased.

For soils low in organic matter ($\leq$ 1.33%), Bowman *et al.*(1965) showed that the adsorption of endosulfan progressively increased as the clay content rose (Table 3-2). Only 36% of the initial amount of α-endosulfan applied was recovered from a soil composed of 4.4% clay following elution with 1,600 ml of distilled water, while from 49 to 87% was eluted at elevated clay contents of 13.4 and 18.4%. Soils having the highest clay content (31.4 to 37.4%) retained 93-100% of the applied α-endosulfan. Similar results were obtained for ß-endosulfan (Table 3-2). Richardson and Epstein (1971) established that within the clay fraction of two soils, 77-90% of the adsorbed endosulfan was found in the smallest size fractions of $<$0.08 to 0.5 μm. These studies suggest that soils high in organic matter and/or clay content will tend to adsorb endosulfan to a greater extent and be more resistant to leaching.

Endosulfan shows a decreased tendency to adsorb to soil as temperature increases. El Beit *et al.* (1981b) observed that the adsorption of α- and ß-endosulfan on Gezira soil decreased from 40% at 2°C to 25% at 45°C. Similarly, a sandy clay loam soil adsorbed approximately 70% of the amounts of each isomer applied initially at 25°C as opposed to only 50% at 45°C. After 70 days at 25°C, 50% remained absorbed versus 10% at 45°C. The authors speculated that the losses at higher temperatures were due to accelerated rates of evaporation and degradation, a conclusion supported in part by the finding (El Beit *et al.* 1981a) that some of the losses from soil columns could be ascribed to conversions to the sulfate and other forms of endosulfan.

3.2.3 Volatilization

Little information is available regarding volatilization of endosulfan from soils and sediments. Based on the high estimated log soil-sorption coefficients of 3.46 for α-endosulfan and 3.83 for ß-endosulfan, little volatilization would be expected to occur from soil (Howard 1991). Bowman *et al.* (1965) established that endosulfan was not easily volatilized from soils having a high organic content. For instance, although 91% of α-endosulfan initially applied to a soil having less than 1% organic content had been volatilized after 8 days at 45°C, only 13% was lost from a soil containing 19% organic matter. Under the same conditions, 65% of ß-endosulfan was lost in low organic soils versus 3% from soil with the highest organic content.

In a marine sediment/water microcosm, substantial amounts of α-endosulfan were lost during the first three days of a 20-day experiment, whereas little volatilization of ß-endosulfan occurred (Cotham and Bidleman 1989). The half-lives of α- and ß-endosulfan in the experiment were estimated at 22 and 8.3 days, respectively.

3.2.4 Hydrolysis and Oxidation

The only degradation product formed by hydrolysis of endosulfan appears to be the alcohol form. Martens (1976) incubated 50 μg of [8,9 - ^{14}C] endosulfan with a sterile nutrient solution at 27°C for 10 days and found that only endosulfan alcohol was formed, and that oxidation of the sulfite group to endosulfan sulfate did not occur. The quantity of endosulfan alcohol formed was directly related to pH. Less than 1% of the endosulfan was hydrolyzed to the alcohol at pH 4.3, versus more than 90% at a pH above 8.0.

3.2.5 pH

pH affects the persistence of endosulfan, apparently leading to more rapid degradation in alkaline soils. El Beit *et al.* (1983) incubated endosulphan isomers with leachates from a loam soil for 130 days at 19-21°C over a pH range from 0.8 to 12.7 and established that there was a progressive reduction in recovery of endosulfan as the pH increased. The recovery rates of both α- and ß-endosulfan declined from approximately 42% at pH 1.6 to 5% at pH 12.0.

3.2.6 Photodecomposition

Both α- and ß-endosulfan can be decomposed to a limited extent by light. El Beit *et al.* (1983) subjected endosulfan (1.02 $mg \cdot mL^{-1}$) in 20 mL of loam soil leachate and 20 g of clay soil to UV irradiation for 168 hours. Both α- and ß-endosulfan exhibited similar rates of photolysis (11% and 8% respectively), in both the leachate and clay soil. Three degradation products were detected, only one of which (endosulfan alcohol) was identified. Archer *et al.*(1972) similarly reported that a seven-day UV irradiation of endosulfan isomers on borosilicate glass produced endosulfan alcohol as the principal degradation product, comprising 22-30% of the original amount. Minor photolysis products, ranging from 1 to 8.5%, included endosulfan ether, α-hydroxyether and endosulfan lactone, while no endosulfan sulfate was produced.

3.2.7 Microbial Transformation

3.2.7.1 *Mixed Cultures of Microorganisms*

Microorganisms in soil have been shown by various studies to metabolize endosulfan. Recoveries of 125 $\mu g \cdot g^{-1}$ of α-endosulfan added to sterilized and unsterilized Gezira soil and incubated at 37°C, were 64% and 51%, respectively, after 42 days (El Beit *et al.* 1981c), while recoveries of ß-endosulfan under identical conditions were 65% and 30% respectively. The significantly greater losses of both isomers from the unsterilized soil support the suggestion that microbial activity was responsible.

Miles and Moy (1979) compared the degradation of endosulfan as well as the sulfate, ether, alcohol, α-hydroxyether and lactone metabolites by incubating them at 20°C for 20 weeks on nutrient media containing a mixed culture of microorganisms derived from a sandy loam soil. Media having microorganisms resulted in more rapid degradation of α- and ß-endosulfan, demonstrated by half-lives of 1.1 and 2.2 weeks respectively versus 12.5 and 5.7 weeks respectively in a sterile control (Table 3-3), and by a greater conversion to endosulfan alcohol (the major degradation product) in the microbial medium. The conversion to alcohol was relatively high, 74 and 77% of α- and ß-endosulfan respectively in the microbial medium versus 31 and 43% in the control, while less than 2% of each isomer was metabolized into endosulfan sulfate in the microbial media. With the exception of endosulfan lactone, half-lives of the remaining metabolites were all substantially less in the microbial medium (indicating greater

degradation) than in the control (Table 3-3). An increase in pH from 6.5 to 7.6 over the course of the experiment complicates the interpretation, however, as alkaline pH levels have been shown to increase the rate of degradation of endosulfan to the alcohol form (Martens 1976)(see Section 3.2.5).

Martens (1977) incubated seven soils having differing proportions of silt, clay, and sand, in columns for 15 weeks following the addition of 10 ppm [8,9 - ^{14}C] endosulfan. For soils kept immersed in water, 2-18% of the initial endosulfan was converted to the alcohol form, 3-8% to the sulfate, and 2-4% to the α-hydroxyether form. Under aerobic conditions, 27-61% of the original endosulfan was oxidized to the sulfate form depending on the type of soil, and less than 3% to the alcohol, lactone and α-hydroxyether forms.

Bacterial degradation of endosulfan has also been shown in experiments simulating sewage treatment plants which demonstrated that organisms tolerated 100 to 250 ppm endosulfan and converted 70%, almost exclusively into the alcohol form, within 7 days (Brooks 1974b).

3.2.7.2 *Bacteria*

Two studies have investigated the role of isolated microorganisms in endosulfan decomposition, showing that various species of bacteria and actinomycetes from the soil are able to metabolize endosulfan, primarily to the alcohol and sulfate forms. Bacteria from a Gezira soil (bacilli, cocci, actinomycetes) were found to metabolize endosulfan in solution following a 42-day incubation at 37°C (El Beit *et al.* 1981c). Both isomers were degraded equally, giving 46% and 49% recovery at the end of the experiment for α- and ß-endosulfan respectively.

Martens (1976) isolated 49 bacterial and 10 actinomycete species from three different agricultural soils and investigated their potential to degrade endosulfan by measuring the amounts lost after 10-day incubations at 27°C following inoculation of nutrient solutions with 50 μg [8,9 - ^{14}C] endosulfan. Fifteen bacteria and three actinomycetes were capable of transforming more than 30% of the endosulfan. Major metabolites were the alcohol and sulfate forms, while minor products included endosulfan α-hydroxyether and two unknowns.

3.2.7.3 *Fungi*

Fungi have been shown by several studies to metabolize endosulfan. A mixed fungal culture derived from Gezira soil consisting of *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp. has been shown to metabolize α- and ß-endosulfan to different extents (El Beit *et al.* 1981c). Following a 42-day incubation at 37°C, more of the α-endosulfan (49%) was degraded, compared to only 25% for ß-endosulfan. Martens (1976) isolated 28 fungi from three agricultural soils and incubated them at 22°C for six weeks in nutrient solutions inoculated with 100 μg [8,9 - ^{14}C] endosulfan. Sixteen isolates showed a capacity to degrade 30% of the added endosulfan. The major metabolite was endosulfan sulfate, but endosulfan alcohol and the α-hydroxyether form were produced in significant amounts.

Aspergillus niger, one of the fungi identified by Martens (1976) as having a high capacity to degrade endosulfan, was isolated from a Gezira soil and added to nutrient flasks containing α- and ß-endosulfan. *A. niger* degraded approximately 33% of each isomer after incubation at room temperature for 10 days (El Zorgani and Omer 1974). Sixty-six percent of α-endosulfan and 69% of the ß-endosulfan were recovered. The major metabolite in this study was endosulfan alcohol, in contrast to the Martens (1976) study where endosulfan sulfate was predominant.

Thus fungi, like bacteria, demonstrate a differential ability to metabolize the two endosulfan isomers. It appears, however, that the rate of fungal metabolism of the α-endosulfan is similar to that of bacteria, while transformation of ß-endosulfan by fungi is somewhat slower.

3.3 FATE OF ENDOSULFAN IN PLANTS

Degradation processes of endosulfan applied to plants have been studied for various species. In such studies, endosulfan residues have been shown to degrade quickly, the half-life calculated to range from 1.95 d to 2.74 d (Pokharkar and Dethe 1981; Das *et al.* 1988). Residues of endosulfan are generally less persistent on plant surfaces and usually less than 0.1 ppm of endosulfan sulphate was detected on leaves and fruits as transformation products (Gupta and Gupta 1979). In a pigeonpea pulse crop and on tomatoes, endosulfan concentrations declined 62 to 64% in the 48 h following the last spray application (Mukerjee 1992; Hughes and Wilson 1972). Similarly, endosulfan residues on jute and grape crops fell 72 to 89% in the 72 h after treatment (Das *et al.* 1988; Singh and Chawla 1979). Most studies concerning the fate of

endosulfan residues on plants have been conducted on crops grown under high temperature regimes of 30 to 43°C (Singh and Chawla 1979; Das *et al.* 1988; Mukherjee *et al.* 1992), however, and behaviour is likely to be different in more temperate conditions.

α- and ß-endosulfan show different behaviour with respect to degradation, the α-isomer degrading more rapidly than ß-endosulfan, but the relative difference appears to vary between studies (Harrison *et al.* 1967; Estesen *et al.* 1979; Singh and Chawla 1979; Pokharkar and Dethe 1981; Das *et al.* 1988; Mukherjee *et al.* 1992). Mukherjee *et al.* (1992) found that the residue of α-endosulfan on a pigeonpea crop decreased by 95% (from 7.53 $mg \cdot kg^{-1}$ to 0.36 $mg \cdot kg^{-1}$) in 15 days, while ß-endosulfan concentration decreased 85% (6.16 $mg \cdot kg^{-1}$ to 0.90 $mg \cdot kg^{-1}$). A larger difference between endosulfan isomers was reported by Harrison *et al.* (1987) for deposits on apple leaves. The time required for endosulfan to be reduced by 98% was three weeks for α- and seven weeks for ß-endosulfan. Pokhardar and Dethe (1981) estimated half-lives of 1.58 and 2.46 d for α- and ß-endosulfan respectively.

Degradation of endosulfan applied to plants produces principally endosulfan sulfate. Other decomposition products such as endosulfan alcohol and endosulfan ether, do not appear on plant surfaces (Cassil and Drummond 1965; Beard and Ware 1969). As concentrations of α- and ß-endosulfan decrease over time, concentrations of endosulfan sulphate increase (Cassil and Drummond 1965; Harrison *et al.* 1967; Estesen *et al.* 1979; Das *et al.* 1988; Mukherjee 1992). The process is temperature-dependent (increasing amounts of endosulfan sulphate forming at higher temperatures) although minimal amounts are formed at 15.5 - 18 °C (Cassil and Drummond 1965). In a study of endosulfan residues on treated apple leaves, no endosulfan sulphate could be found immediately after spraying, while one week later, residues of α-and ß-endosulfan and endosulfan sulfate were present in roughly equal amounts. After three- and seven-week intervals, 75% and 90% respectively of the residue was endosulfan sulphate (Harrison *et al.* 1967). Endosulfan sulphate thus appears to be more persistent than endosulfan (Harrison *et al.* 1967).

Formation of endosulfan sulfate from endosulfan appears to require UV irradiation together with a moisture-containing substrate. Laboratory experients performed by Harrison *et al.* (1967), in which α- and ß-endosulfan were incubated with apple extracts in the light and dark, demonstrated the formation of endosulfan sulphate only in the presence of light, suggesting that UV irradiation was responsible. Direct experimental exposure of technical Thiodan (an endosulfan formulation)

to UV light did not produce endosulfan sulfate, but the compound was formed when Thiodan was exposed in glycerol solution. It thus appears UV and a mosture-containing substrate are necessary for the degradation to endosulfan sulphate to proceed (Harrison *et al.* 1967).

Drying endosulfan-treated alfalfa for 10 days under different conditions, resulted in different loss rates of endosulfan, showing reductions of 48% in the dark, 67% under UV light, and 82% in sunlight (Archer 1972). The concentration of endosulfan sulfate increased in all three treatments, but the most marked increase occurred in the dark.

3.4 ENVIRONMENTAL CONCENTRATIONS

3.4.1 In Canada

Various Canadian studies have measured endosulfan and its isomers in water, bottom sediments and precipitation.

3.4.1.1 *Great Lakes Basin*

In a 1979-80 Environment Canada study of organic contaminants in suspended sediment of the Niagara River, it was reported that endosulfan was the sixth most commonly observed contaminant after PCB, DDT, mirex, chlordane and methoxychlor (Warry and Chan 1981). α-endosulfan was detected in 10 of the 41 samples at concentrations ranging from 2 to 18 $\mu g \cdot g^{-1}$ (average of measured concentrations, 9.5 $\mu g \cdot g^{-1}$). No ß-endosulfan was identified, and as only α-endosulfan was detected, it was proposed that the inputs were of recent origin. The authors concluded that suspended sediments of the Niagara River provide a continuing source of PCBs and pesticide residues to Lake Ontario.

As part of a study on the effects of agricultural land use on quality of water entering the Great Lakes, 949 stream samples from 11 agricultural watersheds in southern Ontario were collected in 1975-1977 (Braun and Frank 1980). Endosulfan was found in 19.3% of the samples. Over 50% of the total organochlorine content was found to be transported during the January to April periods in which conditions were characterized by spring thaws, low ground cover and high suspended solids. Concentrations and unit loadings to the lakes for endosulfan ranged from 0 to 89.9 mg/ha/yr. The water quality objective of 3 $\mu g \cdot L^{-1}$ proposed for endosulfan by the

International Joint Commission was exceeded in 14% of the samples, the highest reported concentration in water being 173 $\mu g \cdot L^{-1}$.

Lower concentrations of endosulfan in waters have been found in other studies. Concentrations from a controlled drainage system in southwestern Ontario ranged from less than 2 to 133 $ng L^{-1}$ and from 4 to 62 $\mu g \cdot kg^{-1}$ in bottom mud (Miles and Harris 1971). Frank and Logan (1988) collected water samples from the mouths of the Grand and Thames Rivers, which drained agricultural land in southern Ontario, from 1981 to 1985. Mean annual concentrations of endosulfan ranged from 0.003 to 0.029 $\mu g \cdot L^{-1}$. Similarly, Kuntz and Warry (1983) reported mean annual concentrations of endosulfan isomers in the Niagara River of 0.0001 $\mu g \cdot L^{-1}$.

When applying pesticides to their crops, farmers use water as the major dilutent and carrier, and draw it typically from farm ponds, wells, ditches, streams or rivers. Sources of contamination to waters have included: surface runoff during storm events, deposits from spray drift, and accidental spills (Frank *et al.* 1990). In a pesticide survey of farm ponds in Ontario, endosulfan was measured in four ponds at a mean concentration of 1.1 ± 0.9 $\mu g \cdot L^{-1}$. Fish kills were reported in three of the four ponds studied. In one of these ponds (endosulfan concentration of 0.56 $\mu g \cdot L^{-1}$) goldfish were found to be dying. In all three cases of fish kills, spraying had occurred up to the edge of the pond.

α-and ß-endosulfan have been observed in precipitation in the Great Lakes region (Stratchan 1979). In 50 samples, α- and ß-endosulfan were observed in 36% and 52% of samples respectively (detection limit 1 $\mu g \cdot L^{-1}$), and were not found in snow. Mean concentrations were 1.5 and 4.9 $\mu g \cdot L^{-1}$ and maxima were 15 and 45 $\mu g \cdot L^{-1}$ for the α- and ß-isomers respectively, highest concentrations occurring in the vicinity of Lake Ontario. The ratio of concentrations of the α- and ß-isomers (about 1:3) was lower than that in the formulation (7:3), suggesting that the endosulfan was older and had degraded. The ratio is compatible with the relative ease of degradation of the two isomers (α-endosulfan degrades more readily). Chan and Perkins (1989) found α- and ß-endosulfan concentrations in precipitation ranging from 0.2 to 9.0 $\mu g \cdot L^{-1}$ and 0.3 to 25.4 $\mu g \cdot L^{-1}$ respectively in the Great Lakes region.

Precipitation can account for a significant proportion of endosulfan loading in aquatic systems. Johnson *et al.* (1988) determined mean loadings of α- and ß-endosulfan respectively between 0.1 and 0.9 $\mu g/m^2/yr$ in two Ontario lake systems. This would result in a loading of between 1 to

9 mg/ha/yr as a result of precipitation. Based on surface run-off loading of 0 to 89.9 mg/ha/yr (Braun and Frank 1980), atmospheric loading could account for 10% of the total found in run-off.

Residues of endosulfan in agricultural soils have generally been detected in the $\mu g \cdot kg^{-1}$ range. Harris and Sans (1971) reported a concentration of 640 $\mu g \cdot kg^{-1}$ endosulfan in soil from one farm in 1969. Samples of organic soil from the Holland Marsh area of Ontario contained from 230 to 500 $\mu g \cdot kg^{-1}$ endosulfan between 1973 and 1975 (Miles *et al.* 1978). Frank *et al.* (1976) found that following applications of 2.2 - 2.5 kg endosulfan/ha (1 or 2 applications anually) to fruit orchards from 1962 to 1975, mean residues of endosulfan in the top 15 cm of soil ranged from 52 to 1,270 $\mu g \cdot kg^{-1}$. From the same study over the 1972 to 1975 period, vineyard soils yielded a mean endosulfan concentration of 70 $\mu g \cdot kg^{-1}$.

3.4.1.2 *British Columbia*

Environment Canada surveyed concentrations of selected pesticides (including endosulfan) in farm ditches that lead to rivers in the Lower Mainland of British Columbia (Wan 1989). A mean endosulfan concentration of 1,530 $\mu g \cdot L^{-1}$ (range 500-2,700 $\mu g \cdot L^{-1}$) was observed in one ditch following spray application even though it was not observed at other times of the year. Endosulfan was consistently found in sediments at all sites at concentrations varying from 2 to 150 $\mu g \cdot kg^{-1}$ and averaging 18.8 $\mu g \cdot kg^{-1}$. There was also evidence to suggest that ensodulfan residues were carried over from previous years in the topsoil (to 5 cm depth) and in the adjacent ditch sediments.

3.4.1.3 *Atlantic Canada*

Endosulfan has been monitored by Environment Canada in the Atlantic region since the early seventies. A search of Environmental Conservation Branch data (NAQUADAT) revealed information on 3,016 samples on which endosulfan analysis had been conducted. Of these analyses, 283 (9%) were primarily quality control spikes and blanks; 2,101 were water samples; and 632 were bottom sediments or biota (Tables 3-4 and 3-5). Quality Control data for the period 1985-1988 indicated that in 71 water samples, median α-endosulfan recoveries were 76% $\pm$ 25% while those for ß-endosulfan were 78% $\pm$ 23%. These values were obtained from the Federal-Provincial survey of municipal drinking water sources and represent both surface and

ground waters from across the region (Léger 1990).

In general, endosulfan has been detected in a small proportion of environmental samples in the Atlantic Region. The compound was found in only 3 (0.6%) water samples (Table 3-4), and both α- and ß-endosulfan occurred in a number of regional precipitation samples during 1988-1990 period (Table 3-4). The ratios of α- and ß-isomers in precipitation indicate weathering and atmospheric transport prior to deposition as proposed in the Canadian Arctic (Welch *et al.* 1991 and Gregor and Gummer 1989) and the Great Lakes region (Stratchan and Huneault 1979 and Johnson 1988). The absence of ß-endosulfan in the two river samples in which endosulfan was detected would tend to indicate an origin in recent product use. Neither α- nor ß-endosulfan were observed in any of the water samples collected during a study of 73 lake bottom sediments in small lakes and ponds in P.E.I. (Clair *et al.* 1987).

Endosulfan has been detected in a small proportion of sediment samples from the Atlantic Region, with the exception of a survey to measure concentrations of pesticides in agricultural basins on Prince Edward Island (Clair *et al.* 1987). In that study, α-endosulfan was observed at concentrations of from 0.001 to 0.005 $mg \cdot kg^{-1}$ (dry weight) in lake bottom sediments (Table 3-5). The absence of ß-endosulfan in lake bottom sediments in that study would suggest recent inputs. In contrast, endosulfan analyses of estuary and river bottom sediments, though not as numerous, indicate that the endosulfan present probably had been weathered.

Though data are limited, there is thus evidence to confirm that weathered endosulfan has been observed in precipitation of the Atlantic Region and that there have been recent inputs of products containing endosulfan into the aquatic environment. Detailed information on use pattern and pesticide inventory would be useful in future environmental monitoring efforts.

3.4.1.4 *Canadian Arctic*

A long-range transport event which deposited thousands of tonnes of fine particulates ('brown snow') on the District of Keewatin in the Central Canadian Arctic was thought to have originated from Asian sources, probably western China (Welch *et al.* 1991). As the result of that event, endosulfan was detected in melted snow at a concentration of 22 $pg \cdot L^{-1}$, leading the authors to conclude that the presence of endosulfan in the Arctic was not exclusively from North American sources. Gregor and Gummer (1989) also documented α-endosulfan concentrations in melted

snow in the Canadian Arctic (ranging from from 0.09 to 1.34 $\mu g \cdot L^{-1}$), while ß-endosulfan was not as frequently detected.

3.4.2 Other Areas

Literature from the 1970s indicate that surface water contamination by endosulfan occurred in the Rhine River of Central Europe, in South Africa and in Australia. In response to a fish kill, monitoring of the Rhine indicated a maximum concentration of 0.70 $\mu g \cdot L^{-1}$ for total endosulfan (Greve and Wit 1971). A second contamination incident happened later in the year (Greve 1972), in which concentrations up to 0.88 $\mu g \cdot L^{-1}$ were measured. In South Africa (VanDyk and Greeff 1977), low concentrations of endosulfan were observed in an irrigated area where fish kills had occurred as a result of accidental spillage. In Australia, endosulfan was one pesticide detected in concentrations sufficiently high to "adversly affect the aquatic environment" (Thoma and Nicholson 1989).

In a recent study, Chandler and Scott (1991) indicated that aqueous concentrations of endosulfan in a tidal creek draining 350 ha of cultivated fields typically ranged from 0.65 to 1.25 $\mu g \cdot L^{-1}$ soon after runoff events, and that endosulfan release was thought to be responsible for large fish kills.

4.0 EFFECTS ON NON-TARGET TERRESTRIAL INVERTEBRATES

Little information is available concerning the effects of endosulfan on non-target soil invertebrates. For earthworms, Hans *et al.* (1990) reported a 24-h LC50 for the earthworm, *Pheretima posthuma*, in soil of 5.01 $mg \cdot kg^{-1}$ endosulfan, and Haque and Ebing (1983) established a 14-d LC50 for the earthworm, *Lumbricus terrestris*, in sandy loam soil of 8.96 $mg \cdot kg^{-1}$ endosulfan. In a laboratory study of non-target microfauna in agricultural soils, Joy and Chakravorty (1991) determined that the toxicity of endosulfan to two soil arthropods was related to the soil type. The mortality of *Xenylla* sp. and *Lancetoppia* sp. progressively decreased from 80 - 100% in sand to 60 - 75% in organic soil. Sandy loam and clay exhibited intermediate toxicity, decreasing in that order. These findings are consistent with greater adsorption of endosulfan to organic and clay fractions of soils (see Section 3.2.2) and probably reflect the lower bioavailability in those soils.

In another field study, Joy and Chakravorty (1991) found that a single application of endosulfan on wheat crops significantly reduced the total number of microarthropods in the soil. The Collembola genera *Cyphoderus* sp. and *Xenylla* sp. failed to recolonize plots 45 d after application, possibly due to a residual toxicity in the soil. Laboratory tests further demonstrated that *Cyphoderus* sp. introduced into the soil 60 days after an endosulfan treatment, exhibited 52% mortality after 72 h, while the mortality of *Xenylla* sp. was 20%.

5.0 EFFECTS ON AQUATIC PLANTS AND INVERTEBRATES

5.1 EFFECTS ON AQUATIC PLANTS

Limited information is available concerning the effects of endosulfan on aquatic plants. Ramachandran *et al.* (1984) exposed six marine macrophytes (*Chaetomorpha linum, Enteromorpha intestinalis, Gracilaria verrucosa, Grateloupia doryphora, Halophila ovalis* and *Halodule uninervis*) to 50 $\mu g \cdot L^{-1}$ endosulfan for six hours at a depth of 1 m *in situ* to determine the effects on photosynthesis and respiration. Net photosynthesis of all six species was reduced only marginally (2-20%) relative to controls. Part of the decrease was attributed to an increase in respiration of 7-12% above controls for *G. verrucosa*, *G. doryphora* and *H. ovalis*. In a similar type of study, Rajendran and Venugopalan (1983) found that a natural phytoplankton population consisting primarily of *Biddulphia sinensis*, *B. mobilensis*, *Chaetoceros cyoartum*, *C. peruvianum*, *Coscinodiscus gigar*, *Rhizosolenia robusta*, *R. alata*, *Skeletonema costatum*, *Thalassiothrix frauenfeldii* and *T. nitzschioides*, exhibited progressively lower rates of photosynthesis relative to those of the controls over a three-hour period, from 74% of controls at 2 $\mu g \cdot L^{-1}$ endosulfan to 25% at 20 $\mu g \cdot L^{-1}$. The EC50 for photosynthesis was 10 $\mu g \cdot L^{-1}$. In contrast, Knauf and Schulze (1973) reported that the photosynthesis, cell division and biomass of the freshwater alga *Chlorella vulgaris* were unaffected by a 5-day exposure to concentrations of endosulfan up to 2,000 $\mu g \cdot L^{-1}$.

Rao and Lal (1987) investigated the uptake and metabolism of endosulfan in the freshwater algae *Aulosira fertilissma* and *Anabaena* sp. over a 48-h period. The accumulation of endosulfan by *A. fertilissma* following exposure to 100, 500 and 1000 $\mu g \cdot L^{-1}$ was initially very rapid with peak concentrations of 178, 296 and 778 $\mu g \cdot g^{-1}$ respectively being attained within 8 to 16 h. By 48 h, concentrations had declined to 49, 75 and 157 $\mu g \cdot g^{-1}$ respectively, primarily because algal biomass had increased. *Anabaena* sp. showed a similar pattern of endosulfan uptake although at

the lowest and highest exposure levels, maximum accumulations were not reached until 40 h. Endosulfan lactone and ether were present at every sampling interval for *Anabaena* sp. indicating that the parent endosulfan was being metabolized (see Sections 5.2.3 and 6.4). For instance, at a concentration of 100 $\mu g \cdot L^{-1}$, peak levels of lactone and ether metabolites of 55.2 and 9.6 $\mu g \cdot g^{-1}$ respectively, were observed.

The studies, which use high exposure concentrations over short periods of time, suggest that it is unlikely that aquatic plants would be adversely affected by endosulfan levels that could be expected in the water column from agricultural runoff or spray drift.

5.2 EFFECTS ON AQUATIC INVERTEBRATES

5.2.1 Acute Toxicity

5.2.1.1 *Marine Invertebrates*

Effects in Water

Marine invertebrates are extremely susceptible to endosulfan. LC50s range from 0.04 $\mu g \cdot L^{-1}$ to 424 $\mu g \cdot L^{-1}$ for a range of species over exposures from 24 to 96 hours (Table 5-1). With the exception of brine shrimp (*Artemia salina*), shrimp appeared to be the most sensitive group, having 24 to 96-h LC50s ranging from 0.04 for a 96-hr exposure to 17.6 $\mu g \cdot L^{-1}$ in a 24-hr test. The 48-h LC50 of *Artemia salina* of 10,000 $\mu g \cdot L^{-1}$ in Table 5-1, however, is three to five orders of magnitude higher than the 48-h LC50s of the other species. Earlier life stages of shrimp appear to be more sensitive to endosulfan than later ones; Joshi and Mukhopadhyay (1990) determined the 24-h LC50s for mysids, post-larvae and juveniles to be 2.4, 7.5 and 17.6 $\mu g \cdot L^{-1}$ respectively. Another crustacean, the crab, *Callinectes sapidus*, was also shown to be sensitive to endosulfan exposure, having a 48-h EC50 (concentration causing mortality or loss of equilibrium in 50% of juvenile crabs tested) of 35 $\mu g \cdot L^{-1}$ (Table 5-1).

Among other groups of marine invertebrates, bivalves also appear to have a pronounced sensitivity to endosulfan, while annelids show more resistance to endosulfan exposure. The clams *Katelysia opima* and *Paphia laterisulca*, have 96-h LC50s of 0.8 and 2 $\mu g \cdot L^{-1}$ respectively (Table

5-1). In contrast, Rao *et al.* (1988) reported a 96-h LC50 of 50 $\mu g \cdot L^{-1}$ for the annelid *Pontodrilus bermudensis*. McLeese *et al.* (1982) conducted a 12-d acute test with *Nereis virens* (as a 96-h test failed to produce any mortality) and established a 288-h LC50 of 100 $\mu g \cdot L^{-1}$.

Zooplankton appear to be most tolerant to endosulfan exposure, having 24-h LC50s ranging from 176 to 424 $\mu g \cdot L^{-1}$ endosulfan for *Eucalanus*, *Lucifer*, *Acartia* and *Sagitta* species (Table 5-1).

Effects in Sediments

Endosulfan in the overlying water has been suggested as the primary source of toxicity to benthic invertebrates in sediment toxicity tests (McLeese and Metcalfe 1980; McLeese *et al.* 1982). McLeese *et al.* (1982), using a sediment low in organic carbon and composed primarily of silt and clay, estimated a 288-h LC50 of 340 $\mu g \cdot kg^{-1}$ endosulfan for the polychaete *Nereis virens*. However, it was observed that after 24 to 96-h the worms emerged from the sediments and did not reburrow, remaining exposed to the surficial water. The 288-h LC50 for *N. virens* in surficial water was determined to be 100 $\mu g \cdot L^{-1}$, the same value as that in seawater alone (Table 5-1), indicating that surficial water was the main source of toxicity. Similarly, in an interlaboratory 28 d acute toxicity test with the polychaete, *Neanthes arenaceodentata*, using sediment with no organic content, the mean 28 d LC50 of the overlying water was 105 $\mu g \cdot L^{-1}$ (Pesch and Hoffman 1983).

Two copepods that dwell at the sediment surface, *Pseudobradya pulchella* and *Nannopus palustris*, exposed to highly organic sediments containing 50,100 and 200 $\mu g \cdot kg^{-1}$ endosulfan showed no significant difference in mortality rates, relative to controls, except for *N. palustris* at the highest concentration, at which 20% of the population died (Chandler and Scott 1991). Endosulfan concentrations in overlying waters were below detectable limits (0.1 $\mu g \cdot kg^{-1}$). The authors attributed the low concentrations to the organic binding capacity of the sediments (see Section 3.2.2). Under the same experimental protocol, the settlement and growth of juveniles of the polychaete *Streblospio benedicti* were significantly adversely affected at all exposure levels. Since *S. benedicti* burrows into and ingests sediments as opposed to the selective feeding of copepods on the sediment surface, the toxicity of endosulfan would appear to be associated with the sediments themselves, rather than with the overlying water. Although the toxicity of the interstitial water in the sediment was not considered in the study, it probably was not a contributing factor, given the highly organic nature of the sediment. Hence, it appears that the

organic content of the sediment is an important factor in determining which compartment will pose the greatest toxic threat to benthic invertebrates.

5.2.1.2 *Freshwater Invertebrates*

The toxicity of endosulfan to freshwater invertebrates varies widely depending on the organism tested. In general, freshwater invertebrates appear to be more tolerant of endosulfan than marine invertebrates, demonstrating 24 to 96-h LC50s ranging from 2.3 to 18,620 $\mu g \cdot L^{-1}$ (Table 5-2). The most sensitive invertebrates based on 48-h LC50s included the amphipod *Gammarus lacustris* (6.4 $\mu g \cdot L^{-1}$), the freshwater prawn *Macrobachium dayanum* (5.3 $\mu g \cdot L^{-1}$) and the stonefly naiad, *Pteronarcys californica* (5.6 $\mu g \cdot L^{-1}$) (Table 5-2). Bivalve molluscs exhibited similar sensitivities with 96-h LC50s ranging from 5.6 $\mu g \cdot L^{-1}$ for *Indonaia caeruleus* to 44 $\mu g \cdot L^{-1}$ for *Lamellidens corrianus*. With the exception of *Chironomus thumini* (48-h LC50 of 2500 $\mu g \cdot L^{-1}$), insect larvae had 48-h LC50s of 21-200 $\mu g \cdot L^{-1}$ endosulfan.

The water flea *Daphnia magna* has generally been shown to have an intermediate sensitivity, having 48-hr EC50s (the concentration at which 50% of the organisms are immobilized) ranging from 62 to 740 $\mu g \cdot L^{-1}$. In contrast, 21-d EC50s for *D. magna* varied from 130 to 170 $\mu g \cdot L^{-1}$ (Nebeker 1982). Some of the variability in these results may have been due to handling stress and reductions in concentrations of dissolved oxygen and endosulfan over time, as this was a 'static' test (with replacement).

Snails, sludge worms and a rotifer were still more resistant to endosulfan than *Daphnia magna*. For several species of snails, 48-h LC50s ranged from 500 to 1,280 $\mu g \cdot L^{-1}$ endosulfan (Table 5-2) and 3,500 $\mu g \cdot L^{-1}$ for the sludge worm *Tubifex tubifex*, while the rotifer, *Brachionus calyciflorus* showed a 24-h LC50 of 5,150 $\mu g \cdot L^{-1}$. Crabs were the most tolerant invertebrates, having 96-h LC50s for several species ranging from 17,400 to 18,620 $\mu g \cdot L^{-1}$ (Table 5-2).

5.2.1.3 *Environmental Factors*

In general, increased temperature leads to greater acute toxicity of endosulfan to invertebrates and fish. With the exception of the damselfly naiad, *Ischura* sp., endosulfan is more toxic to invertebrates at higher temperatures (Table 5-3). For example, the 96-h LC50 of the crab *Oziotelphusa senex senex* declined from 28.6 $\mu g \cdot L^{-1}$ at 12 $^{\circ}$ C to 12.2 $\mu g \cdot L^{-1}$ at 38 $^{\circ}$C. Similarly,

the 96-h LC50 for the bivalve *Lamellidans marginalis* decreased from 40 $\mu g \cdot L^{-1}$ at 19-24 °C to 6 $\mu g \cdot L^{-1}$ at 28-31 °C, reflecting a seven-fold increase in toxicity for an increase in temperature of 7 to 9 °C.

5.2.2 Bioaccumulation and Depuration

Information on bioaccumulation, distribution in tissues, and depuration of endosulfan by invertebrates is restricted to studies on marine species and concerns primarily bivalve molluscs. Roberts (1972) monitored uptake during exposure of the common mussel, *Mytilus edulis*, to concentrations of 100, 500 and 1000 $\mu g \cdot L^{-1}$ endosulfan in a flow-through system for 112 days followed by a 58-day depuration period. Mussels at all concentrations displayed rapid uptake in the first 30 days. At a concentration of 100 $\mu g \cdot L^{-1}$, the amounts of accumulated endosulfan only increased slightly after 30 days, and peaked at 60 days. In contrast, uptake continued to the end of the exposure period (112 d) in animals exposed to higher concentrations. Tissue concentrations at the end of the exposure period were 1.7, 5.5 and 8.1 $\mu g \cdot g^{-1}$ wet weight endosulfan, representing bioconcentration factors (BCFs) of 17.6, 11 and 8.1 for the exposure concentrations of 100, 500 and 1000 $\mu g \cdot L^{-1}$ respectively. Following exposure to clean water, tissue concentrations fell in all groups. At the lowest exposure concentration, tissue levels declined about 50% in the first two weeks of depuration (from 1.7 to 0.8 $\mu g \cdot g^{-1}$) and remained unchanged until the end of the experiment. At the higher concentrations, tissue levels dropped rapidly in the 14-day period following the cessation of exposure, followed by a gradual decline thereafter. For example, in the mussels having the highest exposure concentration (1000 $\mu g \cdot L^{-1}$), tissue levels decreased from 8.1 $\mu g \cdot g^{-1}$ at 112 days to 2.8 $\mu g \cdot g^{-1}$ at 126 days and finally to 1.7 $\mu g \cdot g^{-1}$ at 170 days. Roberts (1972) speculated that the initial rapid fall in residue levels upon transfer to clean water was due to the loss of endosulfan adsorbed on particulate matter in the gut.

Ernst (1977) measured uptake of endosulfan in *M. edulis* exposed to a mixture of seven compounds, each at an initial concentration of 2 $\mu g \cdot L^{-1}$. An equilibrium tissue concentration of 84 $\mu g \cdot g^{-1}$ wet weight endosulfan (BCF = 600) was achieved after 50 hours exposure. Ernst's (1977) calculated BCF is probably an overestimate as it was based on the lowest water concentration measured, 0.14 $\mu g \cdot L^{-1}$.

Rajendran and Venugopalan (1991) investigated the uptake and distribution of endosulfan in the gill, mantle, adductor muscle, foot, and remaining tissues of the oyster, *Crassostrea madrasensis*,

and the clam, *Katelysia opima* following ten-day experiments with exposure concentrations ranging from 0.14 to 1.42 $\mu g \cdot L^{-1}$ endosulfan. The resulting accumulations of endosulfan were low in all tissues. For example, at the lowest exposure concentration (0.14 $\mu g \cdot L^{-1}$), the adductor muscle and foot of *C. madrasensis* accumulated endosulfan residues of 11.1 (BCF=80) and 9.8 $ng \cdot g^{-1}$ (BCF=70) wet weight respectively. *K. opima* exposed to the same endosulfan concentration, accumulated concentrations of 9.4 $ng \cdot g^{-1}$ (BCF=67) and 8.8 $ng \cdot g^{-1}$ (BCF=61) in the adductor muscle and foot respectively.

Haya and Burridge (1988) subjected the polychaete worm, *Nereis virens*, to concentrations of endosulfan averaging 61 $\mu g \cdot L^{-1}$ in water (without a sediment phase) under normal and low-oxygen conditions. After 96 hours, endosulfan continued to be accumulated by both groups. Those organisms exposed to low oxygen accumulated more endosulfan—4,480 μg endosulfan.g^{-1} lipid versus 1,630 μg endosulfan.g^{-1} lipid under normal oxygen conditions. The difference was attributed to the higher ventilation rate of the hypoxic worms. Following a 336-hour depuration period, both groups had cleared virtually all of the endosulfan from their tissues.

In a single study of the bioconcentration of endosulfan by crustaceans, Schimmel *et al.* (1977) exposed the grass shrimp *Palaemonetes pugio* to sublethal concentrations of endosulfan of 0.16 and 0.40 $\mu g \cdot L^{-1}$ in a 96-h test. Whole body BCFs of 81 and 164, respectively, were reported.

To accurately determine the bioconcentration potential of an organism using the steady state approach, measurements of the chemical residue concentrations must be made over a sufficient period of time to ensure that equilibrium conditions exist (Bishop and Maki 1980). Furthermore, the exposure concentration should be maintained at a constant level, typically by using flow-through systems. Although these conditions were not always met in these studies, it appears, based on the limited data available on marine invertebrates, that endosulfan is not concentrated significantly, and that it can be largely eliminated from the tissues. The lack of bioaccumulation studies on freshwater invertebrates, however, permits no such conclusions to be drawn from these studies for aquatic invertebrates in general (see Section 6.3 for a discussion of bioaccumulation and depuration of endosulfan in fish).

5.2.3 Metabolism of Endosulfan

There is a paucity of information concerning the metabolism of endosulfan by invertebrates in the aquatic environment. Knauf and Schulze (1973) determined the acute toxicity of endosulfan and its known metabolites to several different invertebrates (Table 5-4). In general, organisms exposed to endosulfan sulphate had 48-h LC50 values similar to those for exposures to the parent compound, indicating that it was equally toxic. The only exception was *Artemia salina,* in which endosulfan sulfate was two orders of magnitude less toxic than endosulfan. The remaining metabolites consisting of the lactone, alcohol, ether and α-hydroxyether forms of endosulfan, were one to two orders of magnitude less toxic than endosulfan itself for the majority of the invertebrates tested. The exception was the larval stage of the midge *Chironomus thumini*, to which the parent compound and the alcohol metabolite were equally toxic, while the ether metabolite was twice as toxic as endosulfan itself.

5.2.4 Effects on Reproduction

Little information is available on the effects of endosulfan on the reproduction of invertebrates. Onset of reproduction in the rotifer, *Brachionus calyciflorus*, was delayed following treatment with concentrations of endosulfan $\geq$ 1,250 $\mu g \cdot L^{-1}$ while an exposure of 3,300 $\mu g \cdot L^{-1}$ shortened the reproductive period (to 6 days versus 10 days for the controls) (Fernandez-Casalderrey 1991b).

Among crustaceans, Nebeker (1982), in a 21-day life-cycle test with *Daphnia magna*, found that the concentration of endosulfan resulting in no effect on the mean number of young per female per day (no observed effect level, NOEL) was in the range of 20 to 75 $\mu g \cdot L^{-1}$. The marine copepod, *Pseudobradya pulchella*, experienced no adverse reproductive effects upon exposure to 50, 100 and 200 $\mu g \cdot kg^{-1}$ endosulfan in sediments (Chandler and Scott 1991). Over 95% of the barren females at the beginning of the experiment were gravid after seven days in both the control and treatment groups. Similarly, there was no difference in clutch size between the control and treated organisms.

Colonization of sediments containing endosulfan by larvae of the polychaete *Streblospio benedicti*, was significantly reduced at all exposure concentrations (Chandler and Scott 1991). While more than 50% of the control larvae settled, metamorphosed into juveniles and constructed

tubes within 24-h, settlement of exposed larvae dropped to 26% at 50 $\mu g \cdot kg^{-1}$ endosulfan and progressively declined at higher concentrations, reaching < 1% at 200 $\mu g \cdot kg^{-1}$ endosulfan. The juvenile worms initially exhibited depressed feeding activity at 50 and 100 $\mu g \cdot kg^{-1}$ endosulfan and within three to four days had stopped feeding altogether. Not surprisingly, these larvae were significantly smaller than the controls at the conclusion of the experiment.

The limited data points out that endosulfan can have very different effects on the reproductive abilities of invertebrates, depending on the species. More research on the toxicity of endosulfan in the sediments and the water column to a variety of different invertebrates is needed, however, before an in-depth assessment of the effects of endosulfan on invertebrate reproduction is possible.

5.2.5 Effects on Respiration

Several studies have investigated the effects of endosulfan on invertebrate respiration. The respiration rate of grass shrimp, *Palaemonetes pugio*, exposed to 0.16 $\mu g \cdot L^{-1}$ endosulfan (60% of the 24-h LC50) for 24-h was not significantly different from that of the controls (Scott *et al.* 1987). Other species, however, have shown reduced oxygen consumption in response to exposure to endosulfan. Kulkarni *et al.* (1989) found that the marine bivalve, *P. laterisulca*, subjected to 0.8 $\mu g \cdot L^{-1}$ endosulfan (40% of the 96-h LC50) for 96 hours, displayed a significantly reduced oxygen consumption relative to controls. Similarly, the freshwater snail, *Pila globosa*, exhibited a depressed uptake of oxygen with a concomitant increase in carbon dioxide concentration in the blood following a 48-h exposure to 430 $\mu g \cdot L^{-1}$ endosulfan (33% of the 48-h LC50).

Vijayakumari *et al.* (1987) investigated the effect of endosulfan on the respiratory pigment, hemocyanin in the freshwater crab, *Oziotelphusa senex senex*. Significant increases in copper concentration in the hemolymph, indicative of elevated levels of hemocyanin, were observed in crabs exposed to 3,800 $\mu g \cdot L^{-1}$ endosulfan (25% of the 96-h LC50) for four days. The authors suggested that a greater need for oxygen caused by endosulfan intoxication had precipitated the increase in hemocyanin levels.

5.2.6 Biochemical Effects

A small number of studies dealing exclusively with marine and freshwater crabs and a species of freshwater snail, have provided information on biochemical effects of endosulfan in invertebrates. Several studies have examined effects of endosulfan on carbohydrate concentration. The marine crab, *Scylla serrata*, subjected to 200 $\mu g \cdot L^{-1}$ and 300 $\mu g \cdot L^{-1}$ endosulfan (33 and 50% of the 96-h LC50 respectively) for 30 days exhibited significantly reduced levels of glycogen in muscle and hepatopancreas, accompanied by a marked increase of glucose in the blood (Ghosh and Shrotri 1992). Similarly, Kumari *et al.* (1987b) determined that a 48-h exposure to 430 $\mu g \cdot L^{-1}$ endosulfan (33% of the 48-h LC50) resulted in a significant increase in blood glucose in the freshwater snail, *Pila globosa*.

Exposure of the freshwater crab *Oziotelphusa senex senex* to 6,200 $\mu g \cdot L^{-1}$ endosulfan (33% of the 96-h LC50) for 15 days resulted in biochemical changes in lipid concentration and composition in hepatopancreas and claw muscle (Rafi *et al.* 1991). The concentration of lipase, the enzyme responsible for metabolizing triglycerides to fatty acids and glycerol, increased markedly above control levels in both tissues, suggesting that lipolysis was underway. Levels of free fatty acids (FFA) increased, while those of glycerol dropped significantly. Rafi *et al.* (1991) suggested that the glycerol was being incorporated with some of the FFA into lipids, reflecting simultaneous lipogenesis and lipolysis. In addition, the total levels of phospholipids (used as energy sources) in both tissues had decreased markedly while those of cholesterol (used in biosynthesis of several compounds) had increased significantly.

Findings in several studies of the effects of endosulfan on protein metabolism in invertebrates are contradictory. Sumathi *et al.* (1989) found that *O. senex senex* subjected to a concentration of endosulfan equivalent to 10% of the 96-h LC50, exhibited significantly increased protein levels accompanied by markedly reduced amounts of free amino acids (FAA) in the hepatopancreas and claw muscle, suggesting that the amino acids were being incorporated into proteins. Contrasting results were found in another study, in which substantially lower concentrations of proteins coupled with significantly higher levels of FAA, were found in the blood of *Pila globosa* (Kumari *et al.* 1987b) following a 48-h exposure to 430 $\mu g \cdot L^{-1}$ endosulfan. Reddy *et al.* (1991) similarly found lower protein concentrations and higher FAA levels in the hepatopancreas, heart, gills, chelate leg muscle and thoracic ganglion of the freshwater crab, *Barytelphusa guerini*, exposed to 6,000 $\mu g \cdot L^{-1}$ endosulfan (33% of the 96-h LC50) for four days.

The elevated activity of protease in all tissues of *B. guerini* (15 to 22% higher than controls) supports the findings of reduced protein levels and higher FAA concentrations.

Effects of endosulfan have also been reported in studies of various enzyme systems in crabs. The enzymes aspartate aminotransferase (AAT), alanine aminotransferase (AlAT) and glutamate dehydrogenase (GDH) displayed significantly greater levels of activity than the controls in both *O. senex senex* (Sumathi *et al.* 1989) and *B. guerini* (Reddy *et al.* 1991) following 96-h exposures to endosulfan. For example, activity levels of AAT, AlAT and GDH in all of the tissues of *B. guerini* showed substantial increases of 28 to 121, 34 to 78, and 19 to 53% respectively. Reddy *et al.* (1991) speculated that the higher activities of AAT and AlAT reflected the transamination of amino acids which would provide keto acids as precursors in the synthesis of essential organic constituents. They also suggested that the greater GDH activity was in response to a need for α-ketoglutarate, an important intermediate in the citric acid cycle, since GDH catalyzes the oxidative deamination of glutamate, generating α-ketoglutarate. Reddy *et al.* (1992) later reported that both the Na^{+}-K^{+} ATPase and the Mg^{++} ATPase enzymes from the chelate leg muscle, hepatopancreas, heart, gills and thoracic ganglia of *B. guerini* were significantly inhibited following a 96-h exposure to 6,000 $\mu g \cdot L^{-1}$ endosulfan (33% of the 96-h LC50). This would suggest a disruption in osmoregulation and energy production (see Section 6.7).

Yadwad (1989) injected the crab, *Paratelphusa hydrodromus*, with repetitive sublethal doses of endosulfan (4 μg/crab/24 h) directly into the haemocoel to establish whether an induction of glutathione S-transferase (GSH S-transferase) and glutathione (GSH) would occur in the hepatopancreas (the conjugation of xenobiotics with reduced GSH and catalyzed by GSH S-transferase, is an important detoxification mechanism). A marked induction of GSH S-transferase activity and GSH levels was observed within 48 h of the initial exposure. In addition, the rate of incorporation of radiolabelled leucine by the hepatopancreas following a single injection of endosulfan was twice that of the controls, suggesting an increase in protein synthesis. Thus, it appears that *P. hydrodromus* has some ability to detoxify endosulfan. It was also noted that the level of prostaglandins in the hepatopancreas of *P. hydrodromus* had increased over a 24 to 48-h period following the initial exposure to endosulfan (Yadwad *et al.* 1990).

Endosulfan may also affect ionic balance in exposed organisms. A substantial decline in the concentrations of sodium, potassium and chloride was noted in the hemolymph, claw muscle and

hepatopancreas of *O. senex senex* following a 96-h exposure to 6,200 $\mu g \cdot L^{-1}$ endosulfan (33% of the 96-h LC50). The change was attributed to a dilution effect caused by an accumulation of water (Rajeswari *et al.* 1988) (see Section 6.7).

5.2.7 Miscellaneous Effects

Endosulfan has been shown to impact growth or byssal attachment in several bivalve mollusc species. Butler (1963) reported that a 96-h exposure to 65 $\mu g \cdot L^{-1}$ endosulfan caused a 50% decrease in shell growth of the oyster, *Crassostrea virginica*, while exposure to endosulfan impaired the byssal attachment process in the mussel, *Mytilus edulis* (Roberts 1975). Concentrations of endosulfan of 450 and 440 $\mu g \cdot L^{-1}$ caused a 50% reduction in the number of mussels byssally attached (EC50) after 24 and 48 h respectively. The response was temperature- and size-dependent; mussels showed greater sensitivity at higher temperatures, and small mussels were more sensitive than larger ones. In terms of the size effects, the 48-h EC50 for 0.25 g and 33 g wet weight mussels was 640 $\mu g \cdot L^{-1}$ and 940 $\mu g \cdot L^{-1}$ respectively. Mussels also developed fewer byssus threads after exposure to endosulfan, the average number of threads per mussel being approximately 1 after a 48-h exposure to 800 $\mu g \cdot L^{-1}$ compared with 9 threads/mussel for the controls. In a comparative study of byssal attachment, the scallop *Chlamys opercularis* was shown to be more sensitive to endosulfan exposure than *M. edulis*. A concentration of 600 $\mu g \cdot L^{-1}$ endosulfan resulted in a 31% reduction in byssal attachment after 96 h in *M. edulis* as opposed to a 72% reduction in *C. opercularis* (Roberts 1975).

5.2.8 *In situ* Effects on Freshwater Invertebrates

Only two field studies on the effects of endosulfan on aquatic invertebrates have been reported. Van Dyk and Greef (1977) introduced 90 mg endosulfan into a stream and measured concentrations in water and densities of invertebrates at six sites downstream from the application point, a distance of approximately 2.7 km. Immediately after the addition, concentrations at the application site and 50 m downstream were 16.9 and 13.4 $\mu g \cdot L^{-1}$ respectively. Three hours later, levels ranged from about 0.4 $\mu g \cdot L^{-1}$ at intermediate downstream sites, to not detectable at the most distant sites. No endosulfan residues were detected at any site seven hours after the application.

The abundance of freshwater invertebrates, comprising large populations of copepoda, ostracoda,

ephemeroptera, chironomids, simuliidae, corixidae, notonectidae, hydrophylidae and hydreanidae, were measured the day before exposure, immediately after, and one and two days later. An immediate drop in numbers occurred at all sites, ranging from 30 to 94% (Table 5-5). A marked difference in recovery occurred, depending on the type of site. Sites located in pools with little or no current showed increased densities after 24 h. Within 48 h, densities had increased, in some cases substantially, to from 50% to 297% of the pre-treatment levels. The furthest downstream sites (which also had significant currents of 0.6 and 0.75 m/s), showed a progressive decline in densities with time, in one case decreasing by 49% of the pre-treatment density on the day of exposure, by 72% at 24 h, and by 82% at 48 h. It would appear that recoveries at pool sites were caused by recruitment through invertebrate drift, while flowing water sites were not recolonized.

Russell-Smith and Ruckert (1981) investigated the concentrations and effects on aquatic invertebrates of aerial applications of endosulfan over three years to control the tsetse fly, *Glossina moritans* in the Okavango delta in Botswana, Africa. The spray program encompassed four to six applications each year at rates of 6-12 g endosulfan/ha, resulting in an average concentration of endosulfan in the water 12 hours after spraying of 1.2 $\mu g \cdot L^{-1}$ (maximum, 4.2 $\mu g \cdot L^{-1}$).

The degree of impact on population levels of aquatic invertebrates varied depending on invertebrate group and year of application, and ranged from no effect to approximately 50% reductions in some cases. In 1976, no difference could be detected in zooplankton populations (60% of which were rotifers) from control and treated lagoons. The high tolerance of rotifers for endosulfan (24-h LC50 of 5,150 $\mu g \cdot L^{-1}$ for the rotifer *Brachionus calyciflorus* (Fernandez-Casalderrey *et al.* (1991a,b)) may explain the observed lack of change. During the following spray season, macrobenthic populations of oligochaetes, chironomid larvae and trichopteran nymphs from treated areas demonstrated significant reductions of up to 50% relative to those from untreated sites, while ephemeropteran nymphs were unaffected. One year later, the population of larval chironomids had rebounded to a level 50% higher than that observed initially, probably as a result of recruitment from untreated areas. In contrast, populations of oligochaetes, trichoptera and ephemeroptera were apparently unaffected by endosulfan during the 1978 pesticide application season. Chironomid abundance in treated areas increased throughout the spray season whereas numbersin the controls declined.

The results from the two *in situ* studies of endosulfan exposure (Van Dyk and Greef 1977; and Russell-Smith and Ruckert 1981) suggest that some invertebrates may be adversely affected by endosulfan initially, but that populations can usually recover within one year. These studies represent direct applications of endosulfan to aquatic environments, in contrast to the typical mode of entry in Canada, whereby endosulfan is applied as an insecticide on crops and enters surface waters through drift and surface runoff. The lack of information concerning concentrations of endosulfan in surface waters immediately following such applications is a significant data gap.

6.0 EFFECTS ON FISH

6.1 MODE OF ACTION

Although organochlorine insecticides have been used for about 40 years, the precise mode of action remains unknown (Gupta and Gupta 1979; CRC 1981). It is thought that the primary mode of action of cyclodiene compounds like endosulfan is as neurotoxicants, which disrupt the release of acetylcholine from presynaptic vesicles (CRC 1981; Brooks and Mace 1987). This results initially in hyperexcitability and increased respiration, followed by loss of equilibrium, convulsions, irregular opercular movements and death. Other secondary effects include biochemical changes in enzymatic activities as well as in carbohydrate, lipid and protein metabolism. In addition, various histopathological effects on different organs and tissues have been observed.

6.2 ACUTE TOXICITY

6.2.1 Marine Fish

Endosulfan is extremely toxic to marine fish. LC50s for 48 and 96-h exposures to endosulfan range from 0.09 to 1.8 $\mu g \cdot L^{-1}$ (Table 6-1). The spot, *Leiostomus xanthurus*, was the most sensitive fish tested (LC50 of 0.09 $\mu g \cdot L^{-1}$) while the mummichog, *Fundulus heteroclitus*, was the most tolerant (LC50 of 1.15 $\mu g \cdot L^{-1}$) based on 96-h tests. The average acute toxicity value for 96-h tests was 0.63 $\mu g \cdot L^{-1}$.

6.2.2 Freshwater Fish

Endosulfan also shows a high toxicity to freshwater fish. Coldwater species display 24- to 96-h LC50s ranging from 0.3 to 13 $\mu g \cdot L^{-1}$ (Table 6-2). The average 96-h LC50 for rainbow trout, *Oncorhynchus mykiss*, is 1.2 $\mu g \cdot L^{-1}$. Warmwater species demonstrate 24 to 96-h acute toxicities from 0.09 to 41 $\mu g \cdot L^{-1}$ (Table 6-2). Among warmwater species which have been studied, the harlequin, *Rasbora heteromorpha*, is the most susceptible while the European eel, *Anguilla anguilla*, is most tolerant. The average 96-h LC50 for species listed in Table 3-2 is 3.9 $\mu g \cdot L^{-1}$.

Concentrations of endosulfan at or above 50 $\mu g \cdot L^{-1}$ have resulted in complete mortality of exposed individuals. Mulla (1967) reported that exposure to 50 $\mu g \cdot L^{-1}$ endosulfan resulted in 100% mortality of largemouth bass, *Micropterus salmoides*, while carp, *Cyprinus carpio*, subjected to 100 $\mu g \cdot L^{-1}$ endosulfan for 48 hours all died (Ludemann and Neumann 1962). Given the wide range of species tested, encompassing both warmwater and coldwater fish, as well as freshwater and marine forms, it is remarkable that endosulfan is so highly and uniformly toxic.

Exposure to lower concentrations may result in some cases in delayed mortality upon return of the fish to clean water (Schoettger 1970), but fish exposed to endosulfan in toxicity tests may also recover. Kleiner *et al.* (1984) found that there was no delayed mortality of fathead minnows (*Pimephales promelas*) or bluegills (*Lepomis macrochirus*) in the 14-day period following a 24 hour acute toxicity test. Furthermore, survivors exhibited a rapid recovery, swimming normally within 24 hours of being transferred to clean water.

6.2.2.1 *Acute Toxicity Versus Life Stage and Size*

Life Stage

Hashimoto *et al.* (1982) determined the 24-h LC50s for several early life stages of the carp, *Cyprinus carpio*, and established that endosulfan became progressively more toxic from the eyed-egg stage to the eight-week-old fry stage (Table 6-3). The eyed-egg was 1,250 times more resistant (24-h LC50 of 2,500 $\mu g \cdot L^{-1}$) than the eight-week-old fry (24-h LC50 of 2 $\mu g \cdot L^{-1}$), while sac fry and floating fry were four to five times less resistant than the egg stage (24-h LC50s of 560 and 410 $\mu g \cdot L^{-1}$ respectively) (Table 6-3). The two-, four- and eight-week-old fry were about two orders of magnitude more susceptible to endosulfan than the sac and floating fry (24-h

LC50s ranging from 1.5 to 2.0 $\mu g \cdot L^{-1}$). One-week-old fry exhibited an intermediate LC50 of 11 $\mu g \cdot L^{-1}$. Hashimoto *et al.* (1982) hypothesized that an incompletely developed central nervous system (CNS) was responsible for the demonstrated resistance of early life stages to endosulfan, and that once the CNS matured the fish became more susceptible.

Shoettger (1970) also found eggs to be tolerant of endosulfan; no adverse effects on hatching success relative to controls could be demonstrated following exposures of 25-hour-old rainbow trout eggs to concentrations of endosulfan ranging from 10 to 50,000 $\mu g \cdot L^{-1}$ for periods of 30 and 120 minutes. This study suggested that the resistance of the egg stage was due to the selective permeability of the chorion.

Size

In later life stages, fish size is a factor in sensitivity to endosulfan exposure, large fish being more resistant to endosulfan than small ones. Eleven-week-old fry of carp *Cyprinus carpio* are about three times the weight of eight-week-old fry and about 2.5 times more resistant to endosulfan exposure (Table 6-3) (Hashimoto *et al.* 1982). Similarly, young adults of *Sarotherodon mossambicus* weighing 5.9 g are 20 times more resistant to endosulfan than 0.1 g, four-week-old fry in a 24-h acute toxicity test (Matthiessen and Logan 1984). Using larger size classes of fish, Singh and Narain (1982) established that catfish, *Heteropneustes fossilis*, weighing 4.8, 11.3, 23.2 and 41.8 g displayed 96-h LC50s of 5.0, 7.3, 10.0 and 14.7 $\mu g \cdot L^{-1}$ endosulfan, respectively. A size dependence of mortality upon exposure to endosulfan has been confirmed in field studies where endosulfan sprayed in waters to control the tsetse fly, *Glossina moritans*, killed primarily small individuals (Cockbill 1979; Matthiessen *et al.* 1982).

The higher metabolic rate of smaller fish relative to that of larger ones, allowing a greater uptake of endosulfan from the water, may be responsible for their heightened sensitivity. For example, Nowak and Ahmad (1989) reported that the total body burden of endosulfan in catfish, *Neosilurus* sp., exposed to 0.7 $\mu g \cdot L^{-1}$ endosulfan for seven days, was highest at 182.8 $\mu g \cdot kg^{-1}$ wet weight in 9.2 g fish and lowest at 60.3 $\mu g \cdot kg^{-1}$ wet weight in 13.6 g individuals.

The lipid content of fish may also affect their susceptibility to endosulfan. Shoettger (1970) reported that the survival times of goldfish, *Carassius auratus*, subjected to 7 $\mu g \cdot L^{-1}$ endosulfan for 20 days, progressively increased as the respective weights climbed from 96 to 337 g.

Analysis of the lipid content of the muscle tissues revealed that the 96 g fish contained 1.2% fat whereas the 337 g individuals had 6.1% fat. Shoettger postulated that the muscle lipids were acting as a reservoir for endosulfan and were serving as a detoxification system, thereby enabling the large fish with the most fat to survive the longest. Further support for this hypothesis was provided by Matthiessen *et al.* (1982) when in a three-day exposure of *Hepsetus odoe* to 1 $\mu g \cdot L^{-1}$ endosulfan, the caudal muscle, viscera, liver and brain tissues of survivors consistently contained higher lipid levels than those that had died (see Section 6.11.3).

6.2.2.2 *Acute Toxicity of Endosulfan Isomers and Formulations*

Of the two isomers of endosulfan (α-endosulfan and ß-endosulfan, Section 1.2), the α-isomer is 21 to 41 times more toxic to fish than the ß-isomer based on 96-h LC50s (Table 6-4). Technical endosulfan, made up of the α- and ß-isomers in a ratio of 70:30 (Maier-Bode 1968), is intermediate in toxicity between the two isomers.

In addition to its chemical properties, the type of formulation of endosulfan to which fish are exposed can affect its toxicity. Endosulfan is formulated as a wettable powder (WP), emulsifiable concentrate (EC), dust (DU) or as granules (GR) having varying percentages of active ingredient (Maier-Bode 1968). Based on 96-h LC50 data from three species of fish (Table 6-5), the most toxic formulation was emulsifiable concentrate (35% endosulfan), followed by technical endosulfan (96% active ingredient) and the dust (contaminant 4% endosulfan) (Table 6-5). No information is available in the literature concerning the acute toxicity to fish of the granular formulation. The emulsifiable concentrate is generally 1.1 to 2.0 times more toxic than technical endosulfan, and 1.3 to 6.4 times more toxic than the dust. This difference is thought to be due to a greater absorption of the insecticide when emulsifiers are present (Maier-Bode 1968); Coats and O'Donnel-Jeffery 1979).

Acetone, used as a solvent for endosulfan in most of the acute toxicity studies listed in Tables 3-1 and 3-2, is unlikely to have contributed to any of the observed toxicity, both due to use of suitable experimental controls (Devi *et al.* 1981; Rao *et al.* 1980) and to its low toxicity to fish (Alabaster 1969; Majewski *et al.* 1978). Similarly, ethanol, used as a solvent for endosulfan in a few of the studies, has a low toxicity to fish (Majewski *et al.* 1978).

6.2.3 Environmental Factors

Fish are more susceptible to endosulfan at higher temperatures. The 96-h LC50 for rainbow trout, *Oncorhynchus mykiss*, a typical coldwater species, increases 1.7 to 2.7 times for an increase in temperature of 8 to 11 °C (Table 6-6). For warmwater species in Table 6-6, endosulfan became 1.2 to 2.1 times more toxic with temperature elevations of 7 to 12 °C.

The only study of pH effects on mortality due to endosulfan exposure (Schoettger 1970), suggested that pH had no effect on endosulfan toxicity at pHs normally found in the natural environment. In that study, white suckers, *C. commersoni* were exposed in replicate tests to 20 $\mu g \cdot L^{-1}$ endosulfan at 19 °C for 24 h at two different pHs, 6.4 and 8.4. Similar mortality (90-100%) was found at both pH levels.

Only two studies have evaluated the effect of hardness on toxicity of endosulfan to fish, both demonstrating no effect. Schoettger (1970) showed that mortality rates for white suckers subjected to 20 $\mu g \cdot L^{-1}$ endosulfan for 24 h at 19° C did not differ at water hardnesses of 45 and 500 $mg \cdot L^{-1}$ $CaCO_3$. Similarly, Ferrando and Andreu-Moliner (1989) determined that the 96-h LC50s at 15 °C for the European eel, *Anguilla anguilla*, at hardnesses of 250 and 600 $mg \cdot L^{-1}$ $CaCO_3$ were essentially the same (38 and 39 $\mu g \cdot L^{-1}$, respectively).

6.3 BIOACCUMULATION AND DEPURATION

The potential of a chemical to accumulate in biological organisms is generally measured by one of two methods. Using the steady-state approach, the bioconcentration factor (BCF) is estimated by measuring the concentration of the chemical in tissue at equilibrium relative to a constant concentration in water, following an appropriate exposure period (Bishop and Maki 1980; Oliver and Niimi 1985). The constant chemical concentration is maintained using a flow-through exposure system, and the exposure period is usually a minimum of 28 days (Macek *et al.* 1969; Bishop and Maki 1980). In the kinetic approach, the BCF is estimated by comparing the rate constant for uptake to the rate constant for depuration, and an equilibrium need not be attained (Bishop and Maki; Oliver and Niimi 1985). Both methods give comparable results for chemicals such as endosulfan which have short half-lives in fish (Oliver and Niimi 1985). In practise, these conditions are not always met, and measurements of bioaccumulation have been made under conditions involving static or short-term exposure (e.g. Schoettger 1970; Rao 1989), in which

an equilibrium had undoubtedly not been attained, and a constant water concentration could not be confirmed. The following discussion deals only with studies which meet reasonable criteria for estimating bioconcentration factors using the steady-state approach.

6.3.1 Marine Fish

Schimmel *et al.* (1977) exposed striped mullet, *Mugil cephalus*, to 0.035 $\mu g \cdot L^{-1}$ endosulfan for 28 days, and calculated BCF's of 2,429 and 2,755 for the edible (muscle and vertebrae) and offal (head and viscera) tissues, respectively. More than 50% of the total body burden was accumulated during the initial 48 h. Almost all of the endosulfan detected was in the sulfate form, suggesting that only preliminary metabolism of endosulfan had occurred. Once the exposure had been terminated, all of the endosulfan residues were cleared from both edible and offal tissues within 48 h.

Rajendran and Venugopalan (1991) subjected *M. cephalus* and *Mystus gulio* to 0.13 and 0.20 $\mu g \cdot L^{-1}$ endosulfan, respectively for 10 days and measured the accumulation in several different tissues. The highest concentrations, which were found in the liver for both species, were 12.55 $ng \cdot g^{-1}$ wet weight for *M. cephalus* (BCF=96.5) and 42.47 $ng \cdot g^{-1}$ wet weight for *M. gulio* (BCF=213). Endosulfan levels in both fish decreased in the following order: liver > brain > kidney > alimentary canal > muscle > gill, with BCFs for the various organs ranging from 10 to 213. Since no depuration period was included in this study, elimination of endosulfan from the tissues could not be determined.

6.3.2 Freshwater Fish

Only two studies have reported uptake and depuration of endosulfan by freshwater fish. Toledo and Jonsson (1992) exposed zebra fish, *Brachydanio rerio*, to 0.3 $\mu g \cdot L^{-1}$ endosulfan for 21 days, followed by a 5-day depuration period. An equilibrium was reached between 14 and 21 days, and a BCF estimated at 2,650. Of the total body burden of endosulfan, 62% was cleared within 5 days of transfer to clean water, yielding a half-life estimate of 4 days. Under identical experimental conditions, Jonsson and Toledo (1993b) estimated a BCF of 11,583 for the yellow tetra, *Hyphessobrycon bifasciatus*, using the kinetic approach, since an equilibrium had not been attained. Following 5 days depuration, 81% of the endosulfan had been cleared, resulting in a half-life estimate of 1.8 days.

Although the data from these studies show a considerable amount of variation, it is apparent that fish do accumulate endosulfan to a significant extent, but that once exposure to endosulfan has ceased, they clear their tissues quite rapidly. Tissue clearance is consistent with the demonstrated ability of fish to metabolize endosulfan into less toxic metabolites and to excrete them (see Section 6.4).

6.4 METABOLISM OF ENDOSULFAN

Organisms can metabolize endosulfan, producing several chemicals including endosulfan sulfate, lactone, alcohol, ether and α-hydroxyether (Knauf and Schulze 1973). With the exception of the sulfate form, the metabolites are all two to four orders of magnitude less toxic than the parent compound (Table 6-7). Because endosulfan sulfate is almost as toxic as endosulfan itself, it is considered to be an intermediate metabolite, whereas the other forms, which are lower in toxicity, are classed as detoxification products.

Endosulfan isomers and metabolites have been detected following experimental exposures, in organs and tissues including the liver, kidney, gallbladder, gut, brain, gills and muscle, in different species of fish (Table 6-8). α- and ß-endosulfan have been detected in virtually all of the tissues examined, while endosulfan sulfate appeared to be the principal metabolite in all species except in *Channa punctata* and *Anabas testudineus* where the ether form predominated. Detoxification products (e.g. endosulfan lactone, alcohol, ether and α-hydroxyether), have been found mainly in the liver, kidney and gallbladder, suggesting that these sites are the primary detoxification centres. Endosulfan ether appears to be the major detoxification product in all species, except *Heteropneustes fossilis* where the alcohol is the primary one (Table 6-8). A limited ability to detoxify endosulfan was demonstrated in the brain and gill tissues of *C. punctata*, *L. rohita* and *Macrognathus aculeatum*. Muscle tissue containing the parent compound and endosulfan sulfate, appeared to be acting as a storage site (see Table 6-8).

The pathways of endosulfan detoxicification and excretion have been investigated in several studies. Rao *et al.* (1980, 1981) proposed that endosulfan was metabolized mainly in the liver and kidneys and eliminated in the bile and through renal excretion. Rao *et al.* (1980) determined that the anterior gut of *Labeo rohita* contained the parent compound together with the sulfate and the alcohol, while the posterior gut contained only the less toxic metabolites, thus confirming the hypothesis that endosulfan metabolites were secreted into the bile (Table 6-8). Schoettger (1970)

also investigated this possibility in an uptake and metabolism study in white suckers subjected to ^{14}C-labeled endosulfan. Highest residues were found in the liver and gut, in the latter due to the presence of labelled compounds in the feces. Schoettger (1970) postulated that the radioactive residues had entered the gut with the bile and proposed a breakdown pathway in which the liver would remove endosulfan from the blood, convert it to an aromatic metabolite and conjugate it with glucuronic acid, which would then be discharged into the bile and finally into the gut. Support for the hypothesis came from experimental studies which showed that endosulfan metabolites were conjugated with glucuronic acid in the bile of white suckers and goldfish (*Carassius auratus*)(Shoettger 1970). Shoettger finally suggested that endosulfan would be initially oxidized to the sulfate, then converted to the alcohol and finally conjugated with glucuronic acid prior to being excreted and passed into the gut via the bile. Further support for excretion of endosulfan in the bile was provided in field observations by Matthiessen and Roberts (1982) in which two species of fish, *Tilapia rendalli* and *Sarotherodon andersoni*, repeatedly exposed to endosulfan through six aerial applications in a spray program for the tsetse fly, exhibited greatly enlarged gall bladders during the spray season.

6.5 EFFECTS ON OXYGEN CONSUMPTION AND BEHAVIOUR

Oxygen consumption in freshwater fish exposed to endosulfan has been shown to vary depending on endosulfan concentration. In the majority of cases, oxygen consumption increased with concentration from sublethal levels of exposure, and decreased at lethal ones (Rao *et al.* 1980; Jawale 1985; Gopal *et al.* 1985). Rao *et al.* (1980) in a 2-h experiment with *Labeo rohita*, established that oxygen consumption at an endosulfan concentration of 3 $\mu g \cdot L^{-1}$ was three times the level of controls (150 versus 50 mg $0_2.kg^{-1}.h^{-1}$ respectively), but at the higher exposure concentration of 7 $\mu g \cdot L^{-1}$ (above the lethal level) was only slightly elevated (75 mg $0_2.kg^{-1}.h^{-1}$). Similarly, Jawale (1985) reported that concentrations of endosulfan below the 24-h LC50 (0.75 $\mu g \cdot L^{-1}$) stimulated oxygen consumption of *Rasbora daniconius* whereas concentrations above the 24-h LC50 reduced it. In contrast, oxygen consumption was lower at all concentrations in *Macrognathus aculeatum* during endosulfan exposures, reaching 140 and 45 mg $0_2.kg^{-1}.h^{-1}$ at concentrations of 3 and 15 $\mu g \cdot L^{-1}$ respectively versus 480 mg $0_2.kg^{-1}.h^{-1}$ for the controls (Rao *et al.* 1981).

Changes in metabolic rate have been shown to parallel changes in level of activity and type of behaviour accompanying the toxic response to endosulfan exposure. At sublethal concentrations

of endosulfan, fish exhibited hyperexcitability and muscular twitching, and gyrating and darting movements (Singh and Srivastava 1981; Gopal *et al.* 1985; Gill *et al.* 1991a). Lethal levels of endosulfan initially induced erratic swimming and surfacing movements, but subsequently led to loss of equilibrium, convulsions, failure to swim and sinking, irregular opercular movements, and finally death (Devi *et al.* 1981; Gopal *et al.* 1981; Verma *et al.* 1982; Gopal *et al.* 1985; Haider and Inbaraj 1986; Dangé 1986; Maheshwari 1988; Bhatnagar *et al.* 1988; Narain and Singh 1990; Jonsson and Toledo 1993a). The hyperexcitability of fish upon initial exposure to endosulfan is consistent with its primary mode of action as a neurotoxicant (CRC 1981; Brooks and Mace 1987).

The behavioural pattern for *Macrognathus aculeatrum* differed from that of other fish, and reflected the different pattern of response of oxygen consumption to endosulfan exposure. Instead of displaying hyperactivity at the lowest concentrations of endosulfan, activity level became reduced, and the species displayed a progressive inactivity at higher concentrations, ending in death. For this species, Rao *et al.* (1981) speculated that endosulfan was acting more like a respiratory poison than a neurotoxicant.

6.6 EFFECTS ON REPRODUCTIVE BEHAVIOUR

Only a single study examined effects of endosulfan on the reproductive behaviour of fish. Adults of *Sarotherodon mossambicus*, an African cichlid which broods young in its mouth, were subjected to 0.5 $\mu g \cdot L^{-1}$ endosulfan for four weeks (Matthiessen and Logan 1984). The breeding behaviour was apparently unaffected, and no pathological signs were observed in the ovaries or testes at the end of the experiment, but the newly-hatched fry died (24-h LC50 of 0.5 $\mu g \cdot L^{-1}$) (see Section 6.2.2.1). In a nine-week study, treated males exposed to 0.6 $\mu g \cdot L^{-1}$ endosulfan displayed a delayed pattern of reproductive behaviour while in treated females, the rate of clutch production was significantly elevated and the length of time that the eggs or fry were retained was significantly reduced. The authors speculated that the delayed breeding behaviour in males may have caused the females to abort their presumably unfertilized clutches.

6.7 BIOCHEMICAL EFFECTS

Investigations of the effects of endosulfan on biochemical processes in fish fall into several broad categories including: studies of induction of enzymes to metabolize foreign chemicals

(xenobiotics); and studies of enzyme activity; carbohydrate, lipid and protein levels in tissues; and thyroid hormones.

6.7.1 Induction of Enzymes for Metabolizing Xenobiotics

Various enzyme systems are present in biological organisms which have the potential to metabolize and detoxify a range of foreign chemicals (xenobiotics) but only one study has examined induction of these systems in fish in response to exposures to endosulfan. Jensen *et al.* (1991) exposed the rainbow trout *Oncorhynchus mykiss* to endosulfan through intravenous injections and exposures to concentrations in water, and monitored induction of specific enzymes. A single injection of technical grade endosulfan into the caudal vein caused significant increases in ethoxyresorufin O-deethylase (EROD) and aldrin epoxidase (AE) (two enzymes in the cytochrome P-450-dependent monooxygenase (MO) system) relative to those of the controls after 24 hours. However, a six-day exposure of rainbow trout to 8.3 $\mu g \cdot L^{-1}$ (a concentration higher than the 96-h LC50) resulted in a significant increase in activity of arylhydrocarbon hydroxylase (AHH), another enzyme in the MO system. Trout subjected to the same concentration of endosulfan for 14 days showed no induction of enzymes in the glutathione-S-transferase (GST) enzyme system (using 1,2-dichloro-4-nitrobenzene (DCNB) and 1-chloro-2,4-dinitrobenzene (CDNB) as substrates). These results suggest that endosulfan may be a mixed-type inducer of enzymes metabolizing xenobiotics in fish. Further research in this area is required to confirm this hypothesis.

6.7.2 Effects on Enzymatic Activity

Endosulfan has been shown to affect the activities of a number of enzymes in different tissues of fish. Na^{+}-K^{+} ATPase and Mg^{++} ATPase activity in the liver, muscle, kidney, brain and gills of *Channa gachua* exposed to 2.1, 3.6 and 5.6 $\mu g \cdot L^{-1}$ endosulfan for 30 days were significantly inhibited at the two highest concentrations, except for Mg^{++} ATPase in brain tissue which was depressed only at the highest concentration (Dalela *et al.* 1978; Sharma 1988). In an *in vitro* study, Yap *et al.* (1975) found that the activities of Na^{+} - K^{+} ATPase and mitochondrial Mg^{++} ATPase of brain homogenates of the bluegill, *Lepomis macrochirus*, in the presence of an endosulfan solution equivalent to 8,500 $\mu g \cdot L^{-1}$, were reduced 32 and 69% respectively, relative to those of the controls. As the Na^{+} - K^{+} ATPase enzyme is involved in active transport processes across the plasma membrane of cells, any inhibition could result in disruptions in

osmoregulation (Davis and Wedemeyer 1971) (see Section 6.8). Depressed mitochondrial Mg^{++} ATPase activity would result in lower amounts of energy being made available to the organism since the enzyme catalyses the synthesis of ATP (Narayan *et al.* 1984).

The activity of succinic dehydrogenase (SDH), a key enzyme in the citric acid cycle, was reduced significantly in the liver, muscle and heart of *Sarotherodon mossambicus* following a 48-hour exposure to 1 $\mu g \cdot L^{-1}$ endosulfan. Similarly, in a long-term study, SDH activity was depressed in the liver, muscle, kidney, brain and intestine of *Channa punctatus* subjected to 0.2 $\mu g \cdot L^{-1}$ endosulfan for 60 days (Sastry and Siddiqui 1983).

Similarly, the activity of malate dehydrogenase (another enzyme participating in the citric acid cycle), was reduced by 33-55% in liver and skeletal muscles of *Clarias batrachus* seven days after exposure to 0.01 $\mu g \cdot L^{-1}$ endosulfan began (Tripathi and Shukla, 1990). The degree of inhibition remained at the same levels throughout the 28-day experiment. Termination of exposure after seven days led to recovery of enzymatic activity to control levels after 28 days. Endosulfan-induced reductions in activities of enzymes involved in the citric acid cycle would probably result in lower amounts of ATP being synthesized, thereby making less energy available to the organism.

Endosulfan has been shown to have variable effects on the alkaline phosphatase enzyme. Stimulated alkaline phosphatase activity was shown in a study of the liver, kidney, gill and ovary tissue of *Punctius conchonius* after 48 hours exposure to a concentration of 20 $\mu g \cdot L^{-1}$ (=96h Tlm) (Gill *et al.* 1990a). Conversely, Arora and Kulshrestha (1985) reported that alkaline phosphatase activity in the intestine of *Channa striatus* subjected to 0.75 and 1.0 $\mu g \cdot L^{-1}$ endosulfan for 30 days had decreased to levels 30% and 15% of those of the controls, respectively. The authors suggested that this effect could have resulted from histological damage observed in the intestine (Jauhar and Kulshrestha 1983) (see Section 6.9). Acid phosphatase activity was observed to have been elevated significantly in the liver, kidney and ovary (Gill *et al.* 1990a) and in the intestine of fish (Arora and Kulshrestha 1985) as the result of endosulfan exposures.

Activity of the glycolytic enzyme lactate dehydrogenase (LDH) was significantly depressed in the liver, kidney and brain of *C. punctatus* after 60-days exposure to 0.2 $\mu g \cdot L^{-1}$ endosulfan (Sastry and Siddiqui 1983) while elevated LDH activity was observed only in the intestine and muscle. Reduced LDH activity was also observed in liver, muscle, gill and heart tissues of

endosulfan-exposed fish (Gill *et al.* 1990a; Tripathi and Shukla 1990). Enzyme activity in the liver and muscle tissues was restored two and three weeks following cessation of the exposure (Tripathi and Shukla 1990). Other enzymes involved in glycolysis such as hexokinase, glucose-6-phosphatase and pyruvate dehydrogenase were significantly inhibited in the liver, kidney and intestine (Sastry and Siddiqui 1983), but elevated activities of hexokinase and pyruvate dehydrogenase were noted in the muscle tissue. It appears that glycolysis is stimulated in muscle only, which would be consistent with the observed endosulfan-induced behaviour of intense, erratic swimming activity (see Section 6.6).

6.7.3 Effects on Carbohydrate, Lipid and Protein Content of Tissues

Sastry and Siddiqui (1983) found that the glycogen contents of liver and muscle tissues of *Channa punctatus* exposed to 0.2 $\mu g \cdot L^{-1}$ endosulfan for 30 and 60 days were significantly lower than those of the controls. The same effect (glycogenolysis) has also been noted in other endosulfan-treated fish (Singh and Srivastava 1981; Murty and Devi 1982; Dangé 1986; Vasanthi and Ramaswamy 1987; Rao 1989; Ganesan *et al.* 1989; Yasmeen *et al*, 1989; 1991). The only exception to this pattern occurred in a study by Gill *et al.* (1991a) in which the glycogen content in the liver, after falling initially, had recovered to the same level as that of the controls after four weeks, while muscle glycogen had also increased markedly. Brain, gill and heart tissues have been shown to exhibit a reduction in glycogen content following an endosulfan exposure (Yasmeen *et al.* 1991; Vasanthi and Ramaswamy 1987).

A significant increase in blood glucose levels (hyperglycemia) accompanied the decline in glycogen content of the muscle and liver tissues has been observed in several studies (Singh and Srivastava 1981; Dangé 1986; Gill *et al.* 1991a), while a marked accumulation of lactic acid in muscle and liver (Yasmeen *et al.* 1989; 1991) and blood (Sastry and Siddiqui 1993; Gill *et al.* 1991a) has been observed in other studies following endosulfan exposure. Pyruvic acid content has also been shown to be significantly reduced in liver and muscle tissue (Yasmeen *et al.* 1991). These effects point to an ensodulfan-induced increase in carbohydrate metabolism. Dangé (1986) suggested that the use of glycogen by the liver and muscles would provide an immediate source of energy in teleost fish under stress. Certainly, the excitable behaviour demonstrated by fish exposed to endosulfan (see Section 6.6) would necessitate the mobilization of energy reserves, such as glycogen.

Reports of changes in lipid livels in the liver and muscle tissues of fish subjected to endosulfan do not present a consistent pattern. Liver lipids in treated fish declined substantially (Murti and Devi 1982; Ganesan *et al.* 1989; Rao 1989) except in *Barbus conchonius* in which there was no change (Gill *et al.* 1991a). Muscle lipids of endosulfan-exposed fish increased in *Catla catla* (Rao 1989), but decreased in *C. punctata* (Murty and Devi 1982) and *B. conchonius* (Gill *et al.* 1991a). Ovaries (Gill *et al.* 1991a) and kidneys (Rao 1989) of treated fish also showed reductions in lipids. In addition, Gill *et al.* (1991a) found that the cholesterol level in the liver tissue of *B. conchonius* subjected to 6.7 $\mu g \cdot L^{-1}$ endosulfan, was elevated significantly above that of the controls.

The effect of endosulfan on protein levels in the liver, kidney, brain and muscle tissue varies depending on the species tested. Gill *et al.* (1991a) documented significantly higher levels of protein in the liver of *Barbus conchonius* following exposure to 6.7 $\mu g \cdot L^{-1}$ endosulfan for four weeks. In contrast, protein contents in the livers of endosulfan-treated *C.punctata*, *Oreochromis mossambicus* and *C. catla* all declined (Murty and Devi 1982; Ganesan *et al.* 1989; Rao 1989), while Swarup *et al.* (1981) found no effect on liver proteins resulting from endosulfan exposure in *Cirrhinus migalas*. Gill *et al.* (1991a) speculated that additional proteins in the liver were necessary to repair cell organelles and tissues that had suffered damage, in addition to the enzymes required to detoxify endosulfan.

Although Gill *et al.* (1991a) and Swarup *et al.* (1981) detected no change in protein levels in muscles of endosulfan-exposed fish, Murty and Devi (1981) found decreased amounts. In kidneys, proteins levels decreased significantly in treated fish (Swarup *et al.* 1981; Rao 1989). Rao *et al.* (1981) established that *Macrognathus aculeatum* subjected to increasing concentrations of endosulfan ranging from 1 to 15 $\mu g \cdot L^{-1}$, excreted progressively lower amounts of nitrogen. Similarly, Scott *et al.* (1987) observed that ammonia excretion was significantly reduced relative to controls in *Fundulus heteroclitus* exposed to 0.60 $\mu g \cdot L^{-1}$ endosulfan for 96 h. Considering that protein catabolism produces ammonia and other forms of nitrogenous wastes, these findings could reflect a reduction in tissue protein concentrations.

The effects of each of the two isomers comprising endosulfan on the glycogen, lipid and protein content of the liver, kidney and muscle of *C. punctata* were investigated by Murty and Devi (1982). At 0.45 $\mu g \cdot L^{-1}$ of α-endosulfan there was a significant increase in: glycogen, lipid and protein in the liver; lipids and protein in the kidney; and lipid in muscle. Exposure to ß-

endosulfan at 11 $\mu g \cdot L^{-1}$ (24 times higher than for the α-isomer) resulted in declines in glycogen level in the kidney, and in glycogen and protein levels in muscle. Glycogen, lipid, and protein levels in the liver were unaffected. From this study, α-endosulfan appears to have a greater effect on these processes, consistent with the relative acute toxicities of these compounds (Section 6.2.2.2).

Endosulfan has also been shown to lead to reductions in serum levels of calcium and magnesium in *Tilapia mossambica* exposed to a concentration of $0.7 \mu g \cdot L^{-1}$ endosulfan for 10 days (Rangaswamy and Naidu 1989). The authors note that the fish exhibited spasms and tremors characteristic of low serum calcium and magnesium concentrations.

6.7.4 Effect on Thyroid Hormones

Tripathi and Shukla (1990) investigated the effects of the thyroid hormone 3,3',5-triido-L thyronine (T_3) on the activities of cytochrome malate dehydrogenase (cMDH), mitochondrial malate dehydrogenase (mMDH) and LDH enzymes in *Clarius batrachus* which had been exposed to 0.01 $\mu g \cdot L^{-1}$ endosulfan for seven days. An intraperitoneal injection of 20 μg/100 g body weight of T_3 stimulated the activities of all three enzymes in the muscle and liver tissue of unexposed fish to levels 1.5 to 2 times those of uninjected controls, but no similar difference with injection was observed for fish exposed to endosulfan. The T_3 injection only restored to normal levels activities depressed by endosulfan exposure.

The effect of endosulfan on circulating thyroxine (T_4) and T_3 levels was examined in female *C. batrachus* subjected to 8 $\mu g \cdot L^{-1}$ endosulfan for 96 h (Sinha *et al.* 1991a). During the vitellogenic and post-vitellogenic phases of the annual reproductive cycle, T_4 levels were significantly elevated while those of T_3 were markedly reduced. In a similar study, Sinha *et al.* (1991b) investigated the effects of endosulfan in 96-h and 16-day experiments on T_3 and T_4 levels in serum and thyroid follicles in the pharyngeal region as well as in the interior and posterior regions of the kidney in *C. batrachus*. No clear pattern emerged although in both prespawning and spawning fish, T_4 levels generally increased while those of T_3 decreased relative to those of the controls. Thus endosulfan may mediate enzyme activity by impacting the activity of the T_3 hormone.

6.8 EFFECTS ON HEMATOLOGY

No clear picture has emerged from several studies on the effects of endosulfan on hematological parameters in fish. In *Clarius batrachus* subjected to sublethal concentrations of endosulfan of 2,6 and 10 $\mu g \cdot L^{-1}$ for 10 days, the effects varied depending on the parameter measured and the concentration to which they were exposed (Gopal *et al.* 1982). At an exposure concentration of 2 $\mu g \cdot L^{-1}$ the number of red blood cells (RBC), concentration of hematocrit, hemoglobin content, and mean corpuscular hemaglobin concentration (MCHC), all increased significantly above control levels while the mean corpuscular volume (MCV) decreased significantly. At 6 $\mu g \cdot L^{-1}$ the hematocrit declined substantially while other parameters did not differ from those of the controls. At the highest concentration, the RBC, hemoglobin and hematocrit were all significantly depressed.

Similar to *C. batrachus*, *Cyprinus carpio* exposed to a sublethal concentration of endosulfan (0.64 $\mu g \cdot L^{-1}$) for 96 h showed significant increases in RBC and hemoglobin content and a decrease in MCV (Naidu *et al.* 1987), but unlike *C. batrachus*, the MCHC decreased. Gill *et al.* (1991b) found that the RBC and hemoglobin levels of *Barbus conchonius* subjected to 6.7 $\mu g \cdot L^{-1}$ endosulfan for four weeks, initially declined after one week exposure, but increased and approached the control levels by the end of the experiment. Of several types of leucocytes examined including small lymphocytes, large lymphocytes, monocytes, thrombocytes, neutrophils and basophils, only the large lymphocytes were significantly higher in concentration than controls after four weeks.

The RBC, hemoglobin, total leucocyte count (TLC) and packed cell volume (PVC) all increased in *Channa punctatus* exposed to sublethal concentrations of endosulfan for 30 days (Abidi and Srivastava 1988). Similarly, the hemoglobin content of the blood in *C. punctatus* was significantly elevated following 30 and 60-day exposures to 0.2 $\mu g \cdot L^{-1}$ endosulfan (Sastry and Siddiqui 1983). Abidi and Srivastava (1988) noted that a possible factor in some of the change was a decreased blood volume in exposed individuals. The authors speculated that what appeared to be increases in the parameters measured were actually higher concentrations resulting from a loss of water from the blood. Dalela *et al.* (1978) have reported that endosulfan is capable of disrupting the activity of Na^+-K^+ ATPase (an enzyme involved in osmoregulation) in *Channa gachua* (see Section 6.7). Since the hematological effects are conflicting and may be attributable to osmoregulatory imbalances, hematological parameters are probably of little use as indicators

of endosulfan poisoning.

6.9 HISTOPATHOLOGICAL EFFECTS

Long-term exposure of various fish species to sublethal levels of endosulfan can produce adverse histological and other effects on many organs including ovaries, testes, liver, intestine and gills.

6.9.1 Ovaries and Testes

Inbaraj and Haider (1988) determined a lack of activity compared with controls for the enzymes ^{5},3ß-hydroxysteroid dehydrogenase and glucose-6-phosphate in the follicular layer of ovaries of adult *C. punctata* exposed to 0.24, 0.36 and 0.72 $\mu g \cdot L^{-1}$ endosulfan for 120 days. These results suggested that steroid genesis was inhibited by endosulfan. The study also showed that oocyte development was arrested at all concentrations of endosulfan. In ovaries from *Cyprinus carpio* treated with 10 $\mu g \cdot L^{-1}$ endosulfan for 36 h, Haider and Inbaraj (1988) found that a maturation step (germinal vesicle breakdown) induced in oocytes by luteinizing hormone was significantly reduced relative to the controls.

Following a 30-day exposure to 0.75 and 1.00 $\mu g \cdot L^{-1}$ endosulfan, testes and ovaries were removed from *Channa striatus* and examined histologically (Arora and Kulshrestha 1984; Kulshrestha and Arora 1984). Testes exhibited ruptured blood vessels and peritoneum, acute necrosis of connective tissue and interstitial cells, a breakdown of seminiferous lobules and failure of spermatogenesis (Arora and Kulshrestha 1984). Ovaries suffered a reduction in the size and number of oocytes, damage to yolk vesicles in maturing and mature oocytes, increases in the number of atretic oocytes and development of interfollicular spaces (Kulshrestha and Arora 1984).

6.9.2 Liver

Gill *et al.* (1991a) demonstrated significant increases over controls in the average weight of the liver relative to body weight of *Barbus conchonius* exposed to 6.72 $\mu g \cdot L^{-1}$ endosulfan over a four-week period. The enlargement was thought to be due to an infiltration of cholesterol, free fatty acids and other lipids as well as proteins (see Section 6.11.3). Histological examination revealed structural damage including hepatic regression and vacuolization together with nuclear pycnosis

(Gill *et al.* 1990b). Toledo and Jonsson (1992) observed lipid accumulation and zonal necrosis in the liver tissue of *Brachydanio rerio* and *Hyphessobrycon bifasciatus* which had been subjected to 0.3 $\mu g \cdot L^{-1}$ endosulfan for 21 days. Chronic exposure of *Gymnocorymbus ternetzi* for 90 days to 0.4 and 0.5 $\mu g \cdot L^{-1}$ endosulfan resulted in a swelling and disruption of the normal arrangement of hepatic cells and hyperplasia and hypertrophy of the Islets of Langerhans (Amminikutty and Rege 1977).

6.9.3 Gills

Studies involving exposures of fish to sublethal levels of endosulfan for periods from 21 to 32 days have documented remarkedly similar histopathological injuries to the gills (Dalela *et al.* 1979; Bhatnagar *et al.* 1992; Toledo and Jonsson 1992). After eight days exposure to 3.5 $\mu g \cdot L^{-1}$ endosulfan, Dalela *et al.* (1979) reported localized necrosis of some cells together with vacuolization of the lamellae and gill filaments, and initial stages of separation of the basement membrane in *Channa gachua*. Bhatnager *et al.* (1992) observed the same effects after 15 days in *Clarias batrachus* subjected to 3.1 $\mu g \cdot L^{-1}$ endosulfan. At the conclusion of both experiments (30-32 days), cells had clumped together, gill lamellae had fused and the basement membrane had separated completely (Dalela *et al.*1979; Bhatnager 1992). Gill tissues of *Brachydanio rerio* and *Hyphessobrycon bifasciatus* exposed to 0.3 $\mu g \cdot L^{-1}$ endosulfan for 21 days also suffered congestion, structural changes to the lamellae, separation of the epithelium, and necrosis (Toledo and Jonsson 1992).

6.9.4 Intestine

Jauhar and Kulshrestha (1983) exposed *Channa striatus* to 0.75 and 1 $\mu g \cdot L^{-1}$ endosulfan for 30 days and examined the intestine histologically for any changes at the cellular level. After two days at the lowest concentration, tips of villi appeared to have been damaged, columnar cells in the intestinal mucosa had become vacuolated and the connective tissue of the submucosa had developed small spaces. These effects became more pronounced as duration of exposure and concentration increased. After 30 days at 0.75 $\mu g \cdot L^{-1}$ endosulfan, the tips of villi were ruptured, the columnar cells had degenerated, and the spaces in the submucosa had increased in number and size (Jauhar and Kulshrestha 1983).

6.10 *IN SITU* ACUTE TOXICITY

6.10.1 Fish Kills Resulting from Accidental Releases of Endosulfan

Fish kills as a result of accidental endosulfan exposures have been reported occasionally in the literature. The source of introduction in such cases, however, (e.g. spillage, overspraying, runoff, drift, etc.) is rarely mentioned. In a massive fish kill which occurred in the Rhine River in June 1969 (Greve and Wit 1971), the maximum concentration of endosulfan measured one day later from several different sites was 0.70 $\mu g \cdot L^{-1}$. Levels declined steadily and were undetectable at 30 days.

In one of several reports of fish kills in Ontario, Frank *et al.* (1990) reported that runoff from agricultural use of endosulfan without buffer zones, entered four ponds, resulting in concentrations ranging from 0.11 to 2.0 $\mu g \cdot L^{-1}$ and fish mortality in three of the four ponds. Concentrations of endosulfan ranging from 0.096 to 0.26 $\mu g \cdot L^{-1}$ in the headwaters of the Thames River near Brodhagen (Perth County) in August 1969, resulted in the deaths of 300 to 400 fish, primarily minnows (*Phoxinus phoxinus*), white suckers (*C. commersoni*) and rock bass (*Ambloplites rupestris*) (Osmond 1969). The endosulfan was thought to have come from the spraying of nearby potato fields.

Rainbow trout were killed in a pond near Simcoe, Ontario in 1972 following aerial application of endosulfan to local tobacco fields (Frank 1972). Although endosulfan was not detected in the water (limits of detection of α- and ß-endosulfan and endosulfan sulfate were 0.001, 0.002 and 0.01 $\mu g \cdot L^{-1}$, respectively), four trout contained residues of 470 $\mu g \cdot kg^{-1}$ total endosulfan in edible tissues.

Johnston and Cheverie (1980) investigated a fish kill and subsequent recovery after the accidental introduction of endosulfan into North Brook, a tributary in the Dunk River system of Prince Edward Island in 1975. Five days after the accident, they surveyed the brook from the point of entry of endosulfan downstream for a distance of 2.8 km, in addition to a 905 m section that they had previously studied. Approximately one month beforehand, the salmonid population (per 100 meters) of the brook was estimated to be 458 brook trout (*Salvelinus fontinalis*) and eight rainbow trout. Following the accident, 1,032 dead and 2 dying brook trout and 2 dead rainbow trout were collected--no other trout were observed in the stream. No determinations of

endosulfan concentrations in the water were carried out. This study concluded, based on a low recovery of tagged fish and the absence of live individuals, that all of the salmonoid population had been killed and that most of the dead and dying fish had been swept out of the study area.

Ten months after the accident, the 905 m study site was surveyed again. Brook and rainbow trout populations were estimated to have recovered to levels of 38 and 73% respectively of three-year averages determined before the spill, but negligible populations of young-of-the-year brook trout and no young rainbow trout were captured. It appeared from age structure determinations that the newly established populations had been recruited from the Dunk River or from areas above the spill site. The authors speculated that endosulfan residues in the sediments may have been toxic to the eggs or fry.

Ernst *et al.* (1991) investigated the toxicity of endosulfan to threespine sticklebacks, *Gasterosteus aculeatus* as a result of off-target deposition of endosulfan from spraying of potato fields in Prince Edward Island. Bowls filled with water from the source pond for the test fish were placed at distances of 3, 10, 30, 100, 150 and 200 m downwind from the edge of a treated field to collect any endosulfan drift. After the spray event, the water was mixed and samples were taken for analysis of endosulfan residues, prior to being used as medium for acute toxicity tests. The concentration of endosulfan decreased from 700 $\mu g \cdot L^{-1}$ at 10 m to 4$\mu g \cdot L^{-1}$ at 200 m, parallel to concentrations measured in drift sample deposit collectors which showed an exponential decrease with distance downwind from the sprayed field (deposition ranging from 27.6 $mg \cdot m^{-2}$ at 10 m to 0.05 $mg \cdot m^{-2}$ at 200 m). Sticklebacks exposed for 24 h to the water from 200 m (concentration of 4 $\mu g \cdot L^{-1}$) suffered 90% mortality. Similarly, in two separate tests using pond water from the outermost sites (100 and 150 m) of two different spray events, the stickleback mortality was greater than 80%. This study points out the necessity of adequate buffer zones (in this case 200 m or more) for aerial spraying near water bodies.

6.10.2 Fish Kills Resulting from Aerial Spray Programs

Applications of endosulfan to watercourses to control tsetse fly in Africa typically result in fish mortalities. A mass mortality of fish occurred over the entire 40 km length of the Dinya River in Nigeria following aerial application of 1000 g/ha of endosulfan to control *Glossina palpalis* (Koeman *et al.* 1978). Similarly, Everts (1983) found that 200 g/ha endosulfan applications to riverine forests along the Upper Volta and Ivory Coast of West Africa from 1978 to 1981

resulted in 100% mortality of local fish populations. Even at a much lower application rate of 14 g/ha, Cockbill (1979) observed dead fish floating on the surface of pools 7.5 to 10.0 cm deep, one to three days after a spray event. Fingerling bream (*Tilapia* sp.) maintained in open tanks on two separate occasions while the spray plane passed overhead, suffered mortalities ranging from 50 to 100% after 51 to 57 hours of exposure to endosulfan (no concentrations measured)(Cockbill 1979).

Direct aerial application of 9.5 $g \cdot ha^{-1}$ endosulfan to waters of the Okavango delta, Botswana, resulted in concentrations ranging from 0.2 to 4.2 $\mu g \cdot L^{-1}$ in a variety of habitats including a marsh, river, and open pool, six to nine hours after spraying (Fox and Matthiessen 1982). Fish mortality was low 12 to 36 h after spraying in an emergent grass swamp habitat but significant in shallow, open pools. To determine the influence of plants and other materials in *in situ* exposures, Fox and Matthiessen (1982) compared the concentrations of endosulfan in open tanks and cages exposed to the same aerial spray (application rate of 9.5 $g \cdot ha^{-1}$), but which contained either 1) swamp water alone; 2) water and uprooted vegetation; or, 3) water, undisturbed vegetation and silt. Concentrations of 4.0, 2.3 and 0.7 $\mu g \cdot L^{-1}$ were found after 24 h in the three treatments respectively. Fish suffered mortalities of 34 and 36% after 24 h in the first two treatments, but not in the latter. These observations suggest that endosulfan may be adsorbed onto vegetation, silt, and other particulate matter, thereby reducing its bioavailability and lowering its toxicity to fish.

6.11 *IN SITU* SUBLETHAL EFFECTS

6.11.1 Effects on Hematology

Only Matthiessen (1981) has conducted an evalutaion of hematological changes in fish during and after endosulfan application in a natural aquatic environment. As in laboratory studies (see Section 6.8) the results appear to vary with species, season, and breeding condition.

Matthiessen (1981) measured four hematological parameters: erythrocyte count, leucocyte count, hemoglobin concentration, and plasma protein level in different species of fish during and after six sequential applications of endosulfan to the Khwai River system in the Okavango delta in Botswana (application rate of 6 to 12 $g \cdot ha^{-1}$) in 1978. Erythrocyte counts increased significantly during the spray season in *Hepsetus odoë* and *Tilapia sparrmanii*, but only marginally in

Serranochromis angusticeps. By the following April, erythrocyte concentrations had returned to pre-spray levels in *H. odoë* and *S. angusticeps*, but were still slightly elevated for *T. sparmanii*. Total leucocyte counts increased significantly in the three species during the spray season. By April, leucocyte levels for *H. odoë* had returned to pre-spray levels while those of *S. angusticeps* and *T. sparmanii* had fallen below their initial values, although the latter difference was not statistically significant. Whole blood hemoglobin concentrations in *T. sparmanii* (the only species in which this parameter was measured), were unaffected by exposures to endosulfan. *H. odoë* experienced a pronounced drop in plasma proteins during the spray season while *T. sparmanii* exhibited significantly elevated levels.

It would appear from these results that only total leucocyte count is consistently affected by endosulfan exposure. Additional research is required to confirm if this could be a reliable indicator of endosulfan toxicity.

6.11.2 Histological Effects in the Brain and Liver

Matthiessen and Roberts (1982) examined brain tissue of *Tilapia rendalli* and liver tissue of two other fish species exposed to a spray application of endosulfan (the same application as in Matthiessen (1981), described in Section 6.11.1). During the spray season, more than 50% of brain samples of *T. rendalli* displayed subependymal edema, meningitis and infiltrates of inflammatory esinophilic granule cells, while 33% showed severe focal encephalitis and 15% exhibited severe, possibly fatal, intracerebral hemorrhage. In the 12-day period following the last spray event, edema and meningitis were still present at low levels; glial scarring (probably as the result of earlier encephalitis) was present in 50% of the fish; while other lesions had disappeared. Forty days post-spray, glial scarring was still present at a low level, but otherwise the tissue appeared healthy (Matthiessen and Roberts 1982). These histopathological effects may have contributed to behavioural changes in fish (hyperactivity and loss of coordination) observed soon after spraying began (Matthiessen and Roberts 1982).

Liver tissues from *Clarius gariepinus* and *Clarius ngamensis* exhibited degenerative liver damage in the month after spraying began, which tended to diminish in prevalance and severity as the spray program progressed (Matthiessen and Roberts 1982). After the first spray event, 82% of sampled individuals suffered focal necrosis, 47% accumulated toxic lipids, 35% showed edema and 12% exhibited generalized necrosis. By the fourth spray cycle, focal necrosis was reduced

to 67% of individuals while edema and toxic lipid accumulation were present in only 11%. All pathological signs had disappeared at the end of the forty-day period following the last spray event. The recovery of the liver during the spray season suggests that biochemical detoxification mechanisms had become active (see Section 6.7). Matthiessen and Roberts (1982) speculated that the initial increase of lipids in the liver was a detoxification mechanism, whereby endosulfan could be accumulated and stored.

6.11.3 Tissue Residues and Lipid Content

In general, endosulfan tends to accumulate to the greatest extent in tissues having the highest lipid content (Matthiessen *et al.* 1982; Nowak and Julli 1991). Matthiessen *et al.* (1982) determined changes in endosulfan concentration and lipid content of the viscera of five fish species during the same aerial spray program and using the same exposure regime as outlined in Matthiessen (1981) (see Section 6.11.1). The minimum mean endosulfan residue for the five species (140 $\mu g \cdot kg^{-1}$ wet weight) was found in species having the lowest fat content in the viscera (2.7%, *Clarius gariepinus* and *C. ngamensis*). Levels increased progressively in parallel to lipid content, reaching a maximum of 1000 $\mu g \cdot kg^{-1}$ in viscera of *Marcusenius macrolepidotus* (lipid content of 49.4%). In *Sarotherodon macrochir*, the endosulfan concentrations increased from lows of < 100 $\mu g \cdot kg^{-1}$ wet weight in the caudal muscle and gut (respective fat contents of 0.8 and 1.9%) to a maximum of 1,600 $\mu g \cdot kg^{-1}$ wet weight in the visceral adipose tissue (lipid content of 42.1%) (Table 6-9) (Matthiessen *et al.* 1982).

Nowak and Julli (1991) made seasonal measurements of endosulfan concentrations and lipid content in liver tissues from *Tandanus tandanus* (catfish), *Cyprinus carpio* (carp), and *Nematolosa erebi* (bony bream), inhabiting rivers that passed through cotton-growing areas where endosulfan was applied in 1988 and 1989. Highest concentrations of endosulfan in the livers of all three species were found in the summer of 1988 (205 to 912 $\mu g \cdot kg^{-1}$ wet weight), when hepatic lipid levels were most elevated (3.4 to 25.9%) (Table 6-10). Endosulfan residues and lipid content dropped during the winter to ≤ 30 $\mu g \cdot kg^{-1}$ wet weight endosulfan and 2.5 to 14.4% lipid respectively, before increasing to intermediate levels during the summer of 1989 (Table 6-10). In addition to the changing fat levels, the low endosulfan concentrations observed during the winter of 1988 probably reflected the depuration and metabolism of endosulfan in the absence of pesticide applications. The lower endosulfan residues in the summer of 1989 relative to those of 1988 were at least partly due to the reduced hepatic lipid levels. Another contributing factor

may have been that the total rainfall during 1989 was only about half of what is was in 1988, making less endosulfan available through runoff.

The liver is also an important repository for endosulfan in the European eel, *Anguilla anguilla*. Specimens from a lake bordered by agricultural lands where endosulfan was used over a three-year period contained up to 1,450 $\mu g \cdot g^{-1}$ wet weight endosulfan (Ferrando *et al.* 1992a). Similarly, endosulfan residues ranging from 5.8 to 88.6 $\mu g \cdot kg^{-1}$ wet weight were detected in liver tissue of *Tandanus tandanus* collected from a river passing through a cotton-growing area where endosulfan was applied in 1986-1987 (Nowak and Ahmad 1989). Levels of endosulfan in the ovaries and in whole fish (muscles, bone and skin) in the same study were 1.4 to 21.5 and 1.4 to 251.4 $\mu g \cdot kg^{-1}$ wet weight endosulfan respectively.

7.0 EFFECTS ON WILDLIFE

This section presents a brief review of current literature on effects of endosulfan on birds, mammals and amphibians. In general, endosulfan is highly toxic to higher vertebrates through oral doses at all life stages and to amphibians at concentrations likely to be found in the environment.

7.1 TOXICITY OF ENDOSULFAN TO BIRDS

In general, recent testing (Hudson *et al.* 1972, 1984) has indicated that endosulfan is more toxic than previously stated in NRCC (1975). Endosulfan is extremely toxic to mallards and starlings (LD50 $\leq$ 40 $mg \cdot kg^{-1}$), and highly toxic to quail and pheasant (LD50 of 41 to 200 $mg \cdot kg^{-1}$), using the wildlife toxicity classification of the U.S. Fish and Wildlife Service (Smith 1987). DeWitt *et al.* (1963) and Hudson *et al.* (1972) found that young ducks are more sensitive than adults to the insecticide. The acute oral toxicity (LD50) values for various bird species are given in Table 7-1.

Measurements of subacute or dietary toxicity (LC50) of endosulfan to several bird species are listed in Table 7-2. Endosulfan in the diet has been shown to be only moderately toxic to bobwhite quail (LC50 201-1,000 $mg \cdot kg^{-1}$ diet) and slightly toxic to mallard, pheasant and Japanese quail (LC50 1,001-5,000 $mg \cdot kg^{-1}$ diet). Hill (1986) found that LC50 values for Japanese

quail were 2,910 $mg \cdot kg^{-1}$ diet and 2,160 $mg \cdot kg^{-1}$ diet for technical grade endosulfan and Thiodan E, respectively. In addition to the direct toxicity of endosulfan to the organism, these tests reflect any inhibitory effect the compound has on food intake.

Gray partridge *Perdix perdix* fed sublethal levels of endosulfan in their diet (5, 25 and 125 $mg \cdot kg^{-1}$ diet) showed no change in reproductive parameters such as number of eggs laid, number of infertile eggs, number of dead embryos, hatching success, and survival of young chicks. Endosulfan impacts on mixed-function oxidase (MFO) enzymes in liver tissues were assessed in the same birds and in embryos from their eggs. Of the eight cytochrome P-450-dependent monooxygenase enzymes measured in adult birds, four were significantly inhibited by endosulfan 15-55%, while endosulfan had no effect on MFO activities in embryos (Abiola *et al.* 1989; 1992).

Direct exposure of developing chick embryos through injection of endosulfan dissolved in corn oil into the yolk, resulted in 100% and 76% mortality at 200 $\mu g \cdot g^{-1}$ egg and 100 $\mu g \cdot g^{-1}$ egg respectively (Dunachie and Fletcher 1969). No teratogenic effects were observed in the surviving embryos.

7.2 TOXICITY OF ENDOSULFAN TO MAMMALS

The acute toxicity of endosulfan to mammals appears to be similar to that in birds. For rats and dogs, LD50 values range from 18 to 220 $mg \cdot kg^{-1}$ (see references in Maier-Bode 1968; and Gupta and Gupta 1979). The LD50 for lab mice was 6.9-13.5 $mg \cdot kg^{-1}$ (Gupta 1976), while rats given oral doses of 10 $mg \cdot kg^{-1}$/day for 15 days showed decreased weight gains compared to controls and 25% mortality (Gupta 1978). Rats dosed at 5 $mg \cdot kg^{-1} \cdot d^{-1}$ did not exhibit any adverse physical effects. In a similar study, mice given an oral dose of 7 $mg \cdot kg^{-1} \cdot d^{-1}$ for 7 days showed no adverse effects while mice given 14 $mg \cdot kg^{-1} \cdot d^{-1}$ died during the third day (Chatterjee *et al.* 1986).

Chronic feeding trials indicate that mammals sustain sublethal impacts at low doses of endosulfan. In a two-year feeding trial with rats, increases in kidney and liver weights were found with endosulfan levels of 100 $mg \cdot kg^{-1}$ feed (Hazelton Lab. 1959, cited in NRCC 1975), but at a lower concentration (30 $mg \cdot kg^{-1}$ feed) no effects were detected. Another study, similarly failed to detect effects in dogs at endosulfan levels in the feed of up to 30 $mg \cdot kg^{-1}$ (FMC 1967, cited in Maier-Bode 1968).

Repeated-dosing studies have been used to investigate possible sublethal effects in rats. Male rats given oral doses of 5 $mg \cdot kg^{-1} \cdot d^{-1}$ endosulfan for 30 days had increased liver, kidney, epidiymis and testes weights (Dikshith *et al.* 1984). The same animals had increased alkaline phosphatase activity in liver and plasma and increased red blood cell counts. Female rats in the same study were given a lesser daily dose of endosulfan (1.5 $mg \cdot kg^{-1} \cdot d^{-1}$ for 30 days). Dosed females had decreased kidney weights and higher activity of liver alkaline phosphatase but lower enzyme activity in plasma. Both groups of rats displayed behavioural effects such as hyperexcitation, tremor, dyspnea, and salivation during the first 3 days of dosing.

After 30 days of endosulfan dosing, the rats were mated with control rats. Endosulfan effects on total number of young produced were dose-dependent: 18% below normal for the dosed females and 23% lower for the dosed males (Dikshith *et al.* 1984). Female rats dosed with 5 and 10 $mg \cdot kg^{-1} \cdot d^{-1}$ showed dose-dependent teratogenic effects, such as skeletal deformities and fetal resorption (Gupta 1978). Endosulfan given orally to rats in single doses > 10 $mg \cdot kg^{-1}$ or repeated doses > 5 $mg \cdot kg^{-1}$ over 7-15 days induced monooxygenase enzymes systems (cytochrome P-450) in the liver (Agarwal *et al.* 1978; Tyagi *et al.* 1984, 1985; Siddiqui *et al.* 1987). Rats fed lower doses of endosulfan did not induce hepatic cytochrome P-450 (Dorough *et al.* 1978, Tyagi *et al.* 1984). Endosulfan injected in mice at 16.6 $mg \cdot kg^{-1} \cdot d^{-1}$ for 5 days caused decreased sperm counts, abnormal sperm morphology and lethal mutations in sperm cells (Pandey *et al.* 1990). An oral dose of 43.3 $mg \cdot kg^{-1}$ administered over two days did not induce mutations in mouse bone marrow cells (Usha Rani *et al.* 1980).

Bioaccumulation studies cited in Maier-Bode (1968) indicate that endosulfan does not bioaccumulate in mammals. In dosing or feeding trials with sheep, pigs and cattle, residues of endosulfan or its metabolites were only found in fat and milk at low levels, while none was found in muscle or organ tissues. Once exposure to endosulfan ceased, residue levels in fat and milk decreased rapidly. Bioconcentration factors were much less than 1 for all tissues in these studies (NRCC 1975). The half-life of radio-labelled endosulfan in rats given a single oral dose (2 $mg \cdot kg^{-1}$) was about 1.6 days (calculated from Table 3, Dorough *et al.* 1978). In the same study, rats fed radio-labelled endosulfan at 5 $mg \cdot kg^{-1}$ diet for 14 days had kidney, liver and visceral fat residues of 3.1, 1.1 and 0.6 $\mu g \cdot g^{-1}$, respectively. The half-lives of radio-labelled endosulfan in kidney, liver and visceral fat once the rats were switched to uncontaminated feed were about 7, 3 and 1 days, respectively. Gorbach *et al.* (1968) fed a single dose of radio-labelled endosulfan to lactating sheep (approx. 0.26-0.30 $mg \cdot kg^{-1}$). After 22 days, 92% of the

administered endosulfan had been excreted in feces and urine. The half-life, calculated from data in Tables 2 and 4 (Gorbach *et al.* 1968), was about 1.7 days. Residues in the liver, large intestine and fat of the sheep 40 days after dosing were 0.02-0.03 $\mu g \cdot g^{-1}$.

7.3 TOXICITY OF ENDOSULFAN TO AMPHIBIANS

The acute toxicity values for frogs and toads are given in Table 7-3. Indian bull frog tadpoles *Rana tigrina* were an order of magnitude more sensitive to endosulfan than any of the toad (*Bufo*) species listed. Gopal *et al.* (1981) calculated the presumable harmless concentration for *Rana tigrina* tadpoles to be 0.00055 mg/L, although this is only slightly less than the 144-hour LD50 determined by Abbasi and Soni (1991).

7.4 *IN SITU* EFFECTS

Several studies dealing with endosulfan impacts on wildlife have investigated tsetse fly (*Glossina* spp.) control programs in Africa (reviewed in Matthiessen and Douthwaite 1985). In Nigeria, a single application of endosulfan at 800-1000 g/ha killed many birds, particularly insectivores, bats, non-target insects, snakes and many fish (Koeman *et al.* 1978). Two bird species may have been exterminated locally. In other countries, several lighter applications were used throughout the pupal period of the tsetse fly. Aerial applications of 6-28 g/ha were repeated 5-6 times over river, wetland and floodplain areas during studies in Zimbabwe, Botswana and Somalia (Cockbill 1979; Douthwaite 1980, 1986). No adverse effects on non-target insects, aquatic invertebrates, tree frogs or caged mice were found but small fish in shallow pools were killed (Cockbill 1979). Species diversity of birds was unaffected by spraying and no species suffered major declines (Douthwaite 1980).

An insectivorous bird, the little bee-eater *Merops pusillus*, showed no change in feeding behaviour or success after endosulfan application in Botswana (Douthwaite and Fry 1982). A similar study of little bee-eaters in Somalia documented local nest failures associated with spraying, but successful re-nesting averted any population impacts (Douthwaite 1986). Pied kingfishers *Ceryle rudis* modified their foraging behaviour markedly after endosulfan spraying of their riparian habitat (Douthwaite 1982). The birds sought out localized fish kills and fed intensely on debilitated fish. No adverse effects were exhibited by the birds after eating the endosulfan-killed fish. Residues of endosulfan and endosulfan sulphate in liver tissues of

kingfishers (0.012 $\mu g \cdot g^{-1}$), and reed cormorants *Phalacrocorax africanus* (0.023 $\mu g \cdot g^{-1}$), collected one to three weeks after aerial spraying ended were much less than comparable levels in their fish prey (0.14-1.00 $\mu g \cdot g^{-1}$) (Douthwaite 1982; Matthiessen *et al.* 1982). This confirmed that endosulfan did not biomagnify in fish-eating birds.

In a pesticide survey of farm ponds in Ontario (Frank *et al.* 1990) endosulfan was measured in four ponds subjected to endosulfan spray. At one of the ponds, which had an endosulfan concentration of 2.0 $\mu g \cdot L^{-1}$, a dog was poisoned in addition to the fish kill which occurred at the site (Frank *et al.* 1990).

Geese that grazed for 17 days in a field sprayed twice with 50% endosulfan wettable powder at 2 lb/100 gal contained residues below detection limits in liver, kidney, stomach contents and body fat, and displayed no poisoning symptoms (Dustan 1965). Przygodda (1961) found that caterpillars poisoned by endosulfan were toxic when fed to nestling great tits *Parus major* and blackbirds *Turdus merula*.

8.0 ANALYTICAL METHODS

In the last decade, tremendous advances have been made in most aspects of analytical chemistry, especially in the field of environmental analysis. The environmental analysis of chlorinated organic pesticides is now done routinely by many laboratories and it explains why few methods have been published on endosulfan analysis in recent years. Most published methods for endosulfan date back to the 1970's and early 1980s and many are now outdated.

All modern analytical methods for chlorinated organic pesticides involve the same general steps with minor variations of techniques or chemicals. Analysis usually begins with an extraction step using various solvents such as acetone, hexane or dichloromethane, and different extraction techniques or apparatus including soxhlets, sonicators and polytrons. Next is some form of chromatographic cleanup using alumina, florisil, silica gel or other adsorbents to help eliminate the bulk of the matrix and interfering material. Quantitation is usually effected by gas chromatography using open tubular capillary columns coupled with electron capture detectors, or the now widely available "benchtop" mass spectrometers and ion trap detectors. Detection limits are highly dependent on sample type and quantity but routinely range from parts per billion to parts per trillion depending on how much effort is required to meet analytical objectives.

Published endosulfan methods range from determination of total chlorine for total endosulfan to colorimetric methods for isomer-specific analysis. Gas-liquid chromatographic (GC) methods are highly convenient, isomer-specific, and can be used for both residue and environmental analyses (Brooks 1974a). A review of gas chromatographic methods for endosulfan was carried out by Zweig and Sherma (1972). Suprock and Vinopal (1987) evaluated four commercially-available Ultra-Bond gas chromatographic columns with electron capture detectors (ECD). They studied 78 pesticides including both endosulfan isomers and the degradation product endosulfan sulfate. Typical clean-up and fractionation procedures can be found in Mills *et al.* (1972), Sans (1968) and Leoni (1971).

8.1 WATER

A rapid screening method for 50 pesticides (including both endosulfan isomers) in water was developed using two solid phase adsorbents in a single column (Benfenati *et al.* 1990). Analysis was performed with gas chromatography-mass spectrometry (GC-MS). Detection limits for a 1 L sample were 10 $ng \cdot L^{-1}$ with recoveries of 92.3% and 89.2% for the alpha and beta isomers respectively. A method for the analysis of the organochlorine kepone was carried out for water and sediment in the presence of several other organochlorines including endosulfan (Saleh and Lee 1978). The method utilized Florisil column chromatography for clean-up and GC-ECD techniques. No limits of detection were presented for endosulfan but fractionation recoveries were greater than 95% for both isomers.

Recently, Raju and Gupta (1991) used a spectrophotometric method to determine endosulfan in river water and soils. A detection limit of 0.05 $mg \cdot L^{-1}$ was obtained for water.

A study was done of procedural steps in analysing organochlorine pesticides and PCBs using dichloromethane and 15% dichloromethane in hexane at pHs 2, 7 and 10 with determinations by GC-ECD for clean ground water and industrial waste water (Millar *et al.* 1981). Both endosulfan isomers were reported to have greater than 94% recoveries with any solvent or at the 3 pHs tested. Recoveries for the degradation product, endosulfan sulfate were also greater than 89%. Seven-day preservation results indicated better recoveries from samples stored at 4°C than at 24°C. However α- and ß-endosulfan both disappeared at pH 10 during the study and endosulfan sulfate displayed less than 10% recovery at all conditions. Wastewater results indicated good spiking recoveries for endosulfan and endosulfan sulfate during time-zero and 7-day studies.

Spiking amounts into 1 L of water were 50, 100 and 310 ng for α-endosulfan, ß-endosulfan, and endosulfan sulfate respectively. This 1981 study concluded that the applicability of the method might be extended through the use of capillary columns, and confirmed earlier work (Eichelberger and Lichtenberg 1971) which indicated significant endosulfan loss from unpreserved river water spikes after the first week of an 8-week preservation study.

A study in British Columbia (Wan 1989) used dichloromethane to extract 2L water samples for organochlorine pesticides. The extract was dried over anhydrous Na_2SO_4, evaporated and solvent exchanged with iso-octane. The extract was analysed by GC-ECD.

Endosulfan has been extracted from water using commercially available 6-mL SPE C_8 cartridges (Cotham and Bidleman 1989). The cartridges were extracted in a Soxhlet apparatus and activated before use with 3 mL of methanol followed by 3 mL distilled water. The 450 mL sample was drawn through the column (25-35 mL/min) and the column eluted with 3 mL 1:1 ethyl ether/iso-octane. Analysis was carried out using GC-ECD and confirmed with GC-MS.

It is apparent that numerous extraction methods have been used for water samples. While dichloromethane is an excellent extraction solvent (high density and efficient), it does pose problems with GC-ECD systems if there is any carry-over into the final extract. Solid phase extraction systems work well but precautions must be taken to ensure that there is no contamination from the adsorbent.

8.2 SEDIMENTS AND FISH TISSUES

In a method to confirm residues of α- and ß-endosulfan by chemical derivatization (Musial *et al.* 1976), direct spiking of soils (20 g dry weight) with endosulfan indicated that the limit of detection was in the range of 0.5 to 1.0 $\mu g \cdot kg^{-1}$. This confirmation compares favorably with a previous derivatization method (Chau 1969) having a limit of 0.02 $mg \cdot kg^{-1}$ in a 10 g sample extract. The chemical confirmation process was further refined (Chau 1972) using a solid matrix so that 0.005 $mg \cdot kg^{-1}$ in a 10 g fish or sediment extract or 0.003 $\mu g \cdot L^{-1}$ in a 2L water extract could routinely be confirmed.

Endosulfan in sediments from a salt water/sediment microcosm was analysed by freeze-drying and subsequent extraction in a Soxhlet apparatus for 14 hr with 60:40 acetone/hexane (Archer

et al. 1972). The extract was cleaned up with a modified Florisil column and with tetrabutyl ammonium hydrogen sulfite to remove elemental sulfur. Analysis was by GC-ECD with MS confirmation.

Raju and Gupta (1991) used a spectrophotometric method to determine endosulfan in river water and soils. A detection limit of 0.25 $mg \cdot kg^{-1}$ was obtained for soils.

One of the complexities of the analysis of biological tissues is the presence of fats and lipids in the extract matrix. These must be removed prior to quantification. One method is to use 1% and 2% acetone in hexane to chromatograph endosulfan isomers and endosulfan sulfate on partially deactivated Florisil (Rao 1981). The method was evaluated on fortified (5 $mg \cdot kg^{-1}$) samples of butter, whole fish, fish tissue and fat bodies of frogs, resulting in recoveries ranging from 76 to 102%. Gel permeation chromatography can also be used to remove fats and high molecular weight lipids from biological samples.

8.3 MISCELLANEOUS MEDIA

A method was described for analysing organochlorine residues in hard red spring wheat (Levi 1972) that used ball-milling with an ethylether-hexane (3 + 97) extractant, Florisil column cleanup and gas chromatographic-electron capture detection. Recoveries for pesticides including endosulfan were greater than 80% for samples fortified at 0.010 and 0.200 ppm.

Both endosulfan isomers have been measured in strawberries using acetone homogenization, Florisil cleanup and GC-electron capture detection (Zanimi *et al.* 1980). Recoveries for α-endosulfan were 91 $\pm$ 14% and for ß-endosulfan 87 $\pm$ 24% (detection limit of 0.04 ng and 0.2 ng respectively). GC-MS has been used to determine α- and ß-endosulfan and endosulfan sulfate in apples and carrots (Wilkes 1981). The mass spectrometry techniques were used for confirmation at fortification levels of 0.1 $mg \cdot kg^{-1}$.

8.4 METHODS USED IN ENVIRONMENT CANADA LABORATORIES–ATLANTIC REGION

Environment Canada, Conservation and Protection, operates regional laboratories in Moncton New Brunswick, Dartmouth Nova Scotia, and St. John's, Newfoundland. Numerous methods

have been used to quantify endosulfan residues but there are many common procedures within the methods such as use of GC-ECD and Florisil column clean-up. In general the regional endosulfan methodologies are as follows:

8.4.1 Water Samples

- sample is preserved with hexane
- dual extraction with hexane
- extract dried over anhydrous Na_2S0_4
- volume reduced
- GC - ECD detection on either packed or capillary columns

For complex water matrices (e.g. wastewater or contaminated samples), a Florisil or silica gel cleanup step is included. The principle advantage of using hexane in the water extraction is that during the sample work-up, there is no need to perform a solvent exchange prior to quantitation on an electron capture detector.

8.4.2 Sediments and Fish Tissues

- sediment or tissue frozen until analysis;
- extraction with 1:1 acetone/hexane using ultrasonic extraction;
- filtration;
- removal of acetone with water back-extraction;
- dual extraction of acetone/water mix with hexane; and,
- hexane extract treated in similar manner to water extract;

If a high sulphur content is observed, a mercury clean-up step is used to remove elemental sulphur.

9.0 CONCLUSIONS AND RECOMMENDATIONS

Although widely used in Canada, endosulfan is extremely acutely toxic to fish and some invertebrates; is capable of being bioaccumulated; and is persistent in soils. Only limited data

from field studies and concentrations of endosulfan in different environmental matrices (e.g. water, soil, and sediments) is available, however, making it difficult to determine the threat that endosulfan poses to the environment.

Although endosulfan tends to dissipate rapidly from the water column, the initial concentration from runoff or drift may be acutely toxic. However, few studies have investigated the relationship between the application of endosulfan and its subsequent fate in terms of off-target drift, presence in runoff, and persistence in soils and on plant surfaces. Where endosulfan is found from one year to the next as a result of runoff from agricultural fields, it is usually at very low concentrations (in the $ng \cdot L^{-1}$ range), generally below levels known to have sublethal effects.

Endosulfan is bioaccumulated significantly in fish, but can be metabolized, and generally can be depurated from tissues once the exposure has ceased. There do not appear to be any studies measuring depuration in fish constantly exposed to endosulfan, however. Data concerning bioaccumulation of endosulfan by invertebrates is limited to a few studies involving marine bivalves. The lack of information on bioaccumulation and metabolism of endosulfan by freshwater invertebrates, makes it virtually impossible to determine the bioaccumulation potential of endosulfan in these groups.

Endosulfan persists in soils for months and in some cases from year to year, primarily in the surface layers, where it is subject to runoff during precipitation events and snowmelt. Studies examining the dissipation of the α and ß isomers of endosulfan in soil and on plant surfaces have shown that the sulfate form is the primary degradation product in these cases. Because the sulfate form of endofulfan is almost as toxic as the parent compound, it poses the same threat to biota.

Little is known about the dynamics of endosulfan in sediments and the possible threat to aquatic life. Research needs to be conducted on the physiochemical reactions that govern the binding/release of endosulfan in the sediments as well as on the lethal and sublethal toxicity of contaminated sediments to benthic invertebrates.

Runoff from soils is thought to be the major route of entry of endosulfan into watercourses. Once in natural waters, most of the endosulfan in the water column appears to be incorporated in sediments, in which concentrations have been shown to be significantly elevated. To date, however, the few studies investigating the effects of sediment toxicity on invertebrates, are

insufficient to evaluate the potential adverse effects of endosulfan in sediments.

Data collected concerning the use patterns of endosulfan in Atlantic Canada are largely confined to a few localized inventories and use studies. Additional information on product use patterns is required for further monitoring efforts.

Information on endosulfan in the literature is centered on laboratory studies. To accurately determine the threat that endosulfan poses to the environment, several knowledge gaps must be addressed primarily through field research:

1. Field research in the past has concentrated mainly on measuring single parameters, such as the concentration of endosulfan on plant surfaces following an application. It is recommended that:

    a) Comprehensive field studies be conducted in which endosulfan residues applied at controlled rates, are measured in several compartments simultaneously (e.g. plant surfaces, soil, runoff, receiving waters and sediments), over an appropriate period of time, and especially after precipitation events, to accurately determine the fate and persistence of endosulfan in the environment. Based on these results, buffer zones should be developed to protect adjacent surface waters.

    b) In examining environmental matrices for endosulfan residues, evidence of fish kills, if any, should be collected together with representative samples of invertebrates and fish for determination of endosulfan residues. The bioaccumulation potential of these organisms can then be evaluated.

2. Laboratory studies should be conducted on representative species of invertebrates and fish to determine bioaccumulation, and potential for metabolism and depuration under continuous exposure to realistic concentrations of endosulfan as determined from field studies.

3. Physiochemical factors governing the binding/release mechanisms of endosulfan in the sediments should be determined.

4. The toxicity of endosulfan in the sediments should be determined in laboratory studies of representative species of invertebrates living on and in the sediments. These toxic threshold levels should be compared to those found in field tests for comparison and evaluation.

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Table 2-1 Endosulfan sold in New Brunswick 1983-1985 (Shanks 1983, 1984 and 1985)

Year	Insecticide	Active Ingredient (A.I.)	Guarantee $g.L^{-1}$	Amount (L)	Amount (kg)	Amount A.I. (kg)
1983	Endosulfan 400EC	Endosulfan	400	88.0	0	35.2
1983	Thiodan	Endosulfan	400	185.5	0	74.2
1984		Endosulfan		452.0	0	180.8
1985		Endosulfan		792	8.0	320.0

Table 3-1 Recovery of endosulfan from soils having different organic matter contents following eight days of incubation at 45°C. (adapted from Bowman *et al.* 1965).

Soil	Soil Composition				% Recovery	
	% Clay	% Silt	% Sand	% Organic Matter	α-endosulfan	β-endosulfan
Lakeland	4.4	2.6	93	<1	9	35
Rutledge I	2.0	4.0	94	7	62	100
Rutledge II	6.0	5.0	89	19	87	97

Table 3-2 Recovery of endosulfan from soils having varying compositions of clay, silt, sand and organic matter following elution with 1,600 mL of distilled water (adapted from Bowman *et al.* 1965).

		Soil Composition				% Recovery	
	Soil	% Clay	% Silt	% Sand	% Organic Matter	α-endosulfan	ß-endosulfan
Lakeland	Sand	4.4	2.6	93	0.42	36	18
Rutledge I	Sand	2.0	4.0	94	6.56	-	-
Rutledge II	Sand	6.0	5.0	89	19.43	-	-
Lynchburg	Loamy Sand	13.4	4.6	82	0.17	49	22
Magnolia	Sandy Loam	18.4	8.6	93	1.33	87	70
Magnolia	Sandy Clay Loam	31.4	6.6	62	0.72	100	95
Greenville	Sandy Clay	37.4	4.6	58	0.57	100	93
Susquehanna	Sandy Clay	34.6	9.4	56	0.77	93	76

Table 3-3 Half-lives of endosulfan isomers and metabolites incubated with a mixed culture of soil microorganisms (adapted from Miles and Moy 1979).

	Time for 50% degradation (weeks)	
	Sterile Control	Microbial Inoculation
α-endosulfan	12.5	1.1
β-endosulfan	5.7	2.2
endosulfan ether	>20	6
endosulfan α-hydroxyether	>20	8
endosulfan sulfate	>20	11
endosulfan alcohol	>20	14
endosulfan lactone	5.5[1]	5.5[1]

[1]hours

Table 3-4 Summary of Environment Canada monitoring data for water samples in the Atlantic Region

Sample Type	Number of Samples	Period Covered	Number of Positives	Date Found	α-endosulfan ($\mu g \cdot L^{-1}$)	β-endosulfan ($\mu g \cdot L^{-1}$)
Stream	813	1971-88	2	Aug 73	0.018 0.007	N.D. N.D.
Lake	458	1971-88	0	-	-	-
Estuary	158	1971-84	0	-	-	-
Marine	1	1971	0	-	-	-
Pond	5	1989	0	-	-	-
Reservoir	24	1987-88	0	-	-	-
Ground Water	303	1985-89	0	-	-	-
Piezometer	48	1989	0	-	-	-
Tile Drain	2	1989	1	Nov 89	0.043	0.076
Precipitation: Jackson, N.S.[1]	289	1980-91	11	July 88 Aug 88 Nov 88 July 89	0.0008 0.0005 N.D. 0.002	0.0007 0.0017 0.0004 0.002
Jackson, N.S.[1]				July 88 Nov 88	0.0008 N.D.	0.0007 0.0004
Kejimkujik, N.S.				Mar 88 Jul 88 Nov 88 July 89 Feb 90	0.001 0.0005 N.D. N.D. N.D.	N.D. 0.0007 0.0004 0.0003 0.001

N.D. = not detected

[1] For a short period there were two collectors at Jackson for the same parameters.

Table 5-1 Acute toxicity of endosulfan to marine invertebrates.

Species	Formulation	Temperature (°C)	Salinity (°/..)	LC50 (ug·L^{-1}) 24-h	48-h	96-h	Reference
Annelids							
Pontodrilus bermudensis (1988)	35EC	30	20	240 (217-256)	100 (92-115)	50 (43-57)	Rao *et al.*
Nereis virens (1982)		9-10				100[1]	McLeese *et al.*
Crustaceans							
Eucalanus sp.	Tech.			176 (162-191)			Rajendran & Venugopalan (1988)
Acartia sp.	Tech.			243 (194-304)			Rajendran & Venugopalan (1988)
Lucifer sp.	Tech.			290 (262-321)			Rajendran & Venugopalan (1988)
Sagitta sp.	Tech.			424			Rajendran & Venugopalan (1988)
Penaeus duorarum pink shrimp	Tech.	25	16			0.04	Schimmel *et al.* (1977)
Crangon septemspinosa shrimp	-	20				0.2	McLeese and Metcalfe (1980)
Penaeus aztecus brown shrimp	-	30			0.4[2]		Butler (1963)
Palaemonetes pugio grass shrimp	Tech. 24EC	23.9 25	20.9 20			1.31 0.25	Schimmel *et al.* (1977) Scott *et al.* (1987)
Crangon crangon brown shrimp		15			10		Portmann and Wilson (1971)
Penaeus monodon tiger prawn (juvenile)	35EC	32	16	17.6	12.2		Joshi and Mukhopadhyay (1990)
Artemia salina brine shrimp	35EC				10,000		NRCC (1975)
Callinectes sapidus crab	-	30			35[3]		Butler (1963)
Molluscs							
Paphia laterisulca (1989) clam	35EC	16-21	29-30.5			2	Kulkarni *et al.*
Katelysia opima clam	35EC	21-23	34			0.8	Mane *et al.* (1983)

[1] 288-h LC50
[2] Concentration in seawater causing mortality or loss of equilibrium in 50% of adult shrimp tested.
[3] Concentration in seawater causing mortality or loss of equilibrium in 50% of juvenile crabs tested.

Table 5-2 Acute toxicity of endosulfan to freshwater invertebrates.

Species	Formulation	Temperature (°C)	LC50 ($\mu g\ L^{-1}$) 24-h	48-h	96-h	Reference
Rotifers						
Brachionus calyciflorus	99% Tech./ acetone	25	5150 (4380-5920)			Fernandez-Casalderrey *et al.* (1991a)
Annelids						
Tubifex tubifex	35 EC	-		3500		Knauf and Schulze (1973)
Crustaceans						
Cladocera						
Daphnia magna	-	20		390[1] (310-490)		Nebeker (1982)
Daphnia magna		20		630[1] (550-720)		Nebeker (1982)
Daphnia magna	-	20		720[1] (580-900)		Nebeker (1982)
Daphnia magna	-	20		372[1] (325-432)		Nebeker (1982)
Daphnia magna	-	20		328[1] (293-371)		Nebeker (1982)
Daphnia magna	-	20		218[1] (91-572)		Nebeker (1982)
Daphnia magna	-	20		282[1] (245-328)		Nebeker (1982)
Daphnia magna	-	20		266[1] (241-296)		Nebeker (1982)
Daphnia magna	-	20		158[1] (137-186)		Nebeker (1982)
Daphnia magna	-	20		378[1] (220-645)		Nebeker (1982)
Daphnia magna	-	20		740 (521-1051)		Nebeker (1982)
Daphnia magna	-	20		280[1] (217-361)		Nebeker (1982)

Cont'd

Table 5-2 (Cont'd) Acute toxicity of endosulfan to freshwater invertebrates.

Species	Formulation	Temperature (°C)	LC50 (µg·L⁻¹) 24 h	48 h	96 h	Reference
Daphnia magna	96.4% tech./ ethanol	10	178 (162-194)	132 (113-151)	52.9 -	Schoettger (1970)
Daphnia magna	96.4% tech./ ethanol	19	68 (54-82)	62 (49-75)	56.0 -	Schoettger (1970)
Daphnia magna	35 EC	-		400		NRCC (1975)
Daphnia magna	tech./tri-ethylene glycol	20		343 (263-447)		Nebeker (1982); Nebeker *et al.* (1983)
Daphnia magna	tech./tri-ethylene glycol	20		271 (226-325)		Nebeker (1982); Nebeker *et al.* (1983)
Daphnia pulex				240		F.W.P.C.A. (1968)
Amphipods						
Gammarus lacustris	tech. 96%	21	9.2 (6.8-12.0)	6.4 (5.0-8.2)	5.8 (4.1-8.1)	Sanders (1969)
Decapods						
Macrobrachium dayanum	35EC	26	6.2 (5.7-6.8)	5.3 (4.4-6.4)	4.1 (3.5-4.7)	Omkar & Murti (1985)
Barytelphusa guerini crab	35EC	-			17780	Reddy *et al.* (1991)
Oziotelphusa senex senex crab	99% tech.	-			18620	Rajeswari *et al.* (1988)
Oziotelphusa senex senex	95% tech.	28			17400 (16000-18300)	Reddy *et al.* (1992)
Insecta						
Ischura sp. damselfly naiads	96.4% tech./ ethanol	8	235 (165-305)	120 (65-180)	71.8 -	Schoettger (1970)
Ischura sp. damselfly naiads	96.4% tech./ ethanol	19	275 (240-310)	175 (135-215)	107 -	Schoettger (1970)
Pteronarcys californica stonefly naiads	tech.	15.5	24 (15-37)	5.6 (3.9-8.1)	2.3 (1.6-3.3)	Sanders and Cope (1968)
Chironomus thumini	35EC	-		2500		Knauf and Schulze (1973)
Aedes aegypti	35EC	-		200		Knauf and Schulze (1973)

Cont'd

Table 5-2 (Cont'd) Acute toxicity of endosulfan to freshwater invertebrates.

Species	Formulation	Temperature (°C)	LC50 ($\mu g \cdot L^{-1}$) 24 h	48 h	96 h	Reference
Enallagma sp.	90% tech./ ethanol	20	28.5	21	17.5	Gopal *et al.* (1981)
Mollusks						
Gastropods						
Aplexa hypnorum	23.5% tech.	24.5	-	-	>1890	Holcombe *et al.* (1983)
Planorbis corneus	35EC	-	-	1000		NRCC (1975)
Limnea stagnalis	35EC	-		1200		NRCC (1975)
Physa fontinalis	35EC	-		500		NRCC (1975)
Pila globosa	-	-		1280		Kumari *et al.* (1987)
Pelecypods						
Lamellidens corrianus	35EC	19-24			44	Mane and Muley (1984)
Lamellidens marginalis	35EC	19-24			40	Mane and Muley (1984)
Indonaia caeruleus mussel	35EC	29-31			5.6	Mane *et al.* (1983)

[1] EC50 - effect concentration resulting in 50% immobilization.

Table 5-3 Effect of temperature on the acute toxicity of endosulfan to freshwater invertebrates.

Species	Temperature (°C)	LC50 (ug·L⁻¹) 48-h	LC50 (ug·L⁻¹) 96-h	Reference
Daphnia magna	10	132	52.9	Schoettger (1970)
	19	62	56.0	
Ischura sp.	8	120	71.8	Schoettger (1970)
	19	175	107.0	
Oziotelphusa senex senex	12	-	-	Reddy *et al.* (1992)
	28	-	17.4	
	38	-	12.2	
Lamellidans marginalis	19-24	-	40	Mane and Muley (1984)
	25-27	-	36	
	28-31	-	6	
Lamellidans corrianus	19-24	-	44	Mane and Muley (1984)
	25-27	-	40	
	28-31	-	17	

Table 5-4 Acute toxicity of endosulfan and metabolites to invertebrates (adapted from Knauf and Schulze 1973).

Species	48 h LC50 ($\mu g \cdot L^{-1}$)					
	35EC	Endosulfan Sulfate	Endosulfan Lactone	Endosulfan Alcohol	Endosulfan Ether	Endosulfan α-hydroxyether
Tubifex tubifex	3,500	2,500	-	40,000	90,000	-
Daphnia magna	400	400	50,000	500	750	250
Artemia salina	10,000	750	>100,000	8,000	20,000	-
Aedes aegypti	200	500	40,000	5,000	2,500	10,000
Chironomus thumini	2,500	-	-	2,500	5,000	-
Planorbis corneus	1,000	3,000	90,000	90,000	90,000	8,000
Limnea stagnalis	1,200	5,000	50,000	10,000	5,000	-
Physa fontinalis	500	750	70,000	-	5,000	-

Table 5-5 Densities of invertebrates (organisms/m^2) in a stream following introduction of 90 mg endosulfan (from Van Dyk and Greef 1977).

Time	Densities (organisms/m^2)					
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
24 h pre-treatment	6,232	20,863	7,852	1,828	6,929	5,140
treatment	4,387	1,337	4,180	1,073	3,559	3,465
24 h post-treatment	17,116	8,492	7,155	1,657	1,958	2,146
48 h post-treatment	11,731	9,977	9,659	5,423	1,261	1,751

Table 6-1 Acute toxicity of endosulfan to marine fish.

Species	Size (g)	Formulation	Temperature (°C)	Salinity (°/°°)	LC50 ($\mu g \cdot L^{-1}$) 24-h	48-h	96-h	Reference
Fundulus heteroclitus mummichog	adults	EC 30	20	20			1.15 (1.13-1.18)	Trim (1987); Scott *et al.* (1987)
Idus melanotus	-	EC 35	-	3		1.8		NRCC (1975)
Leiostomus xanthurus U.S.Fish spot	juvenile	-	22	26		0.6		Wildlife Serv. (1963)
	3.87 cm	tech./acetone	25	18			0.09 (0.06-0.11)	Schimmel *et al.* (1977)
Mugil curema (1963) white mullet	-	-	29	-		0.6		Butler
Mugil cephalus striped mullet	2.71 cm	tech./acetone	23.7	15.3			0.38 (0.35-0.47)	Schimmel *et al.* (1977)
Lagodon rhomboides pinfish	2.97 cm	tech./acetone	24.3	16.4			0.30 (0.25-0.47)	Schimmel *et al.* (1977)
Cyprinidon variegatus sheepshead minnow	-	-	23	31			1.21	Bengtson *et al.* (1984)

Table 6-2 Acute toxicity of endosulfan to freshwater fish.

Species	Size (g)	Formulation	Temperature (°C)	LC50 ($\mu g \cdot L^{-1}$) 24-h	48-h	96-h	Reference
Oncorhynchus mykiss rainbow trout	1.0-1.8	tech./ethanol	1.5	5.9 (5.2-6.6)	2.1 (1.4-2.8)	0.8	Schoettger (1970)
	1.0-1.8	tech./ethanol	10	2.1 (1.5-2.7)	1.1 (0.7-1.5)	0.3 -	Schoettger (1970)
Oncorhynchus mykiss rainbow trout	0.6-1.5	tech./acetone	1.6	13 (11.15)		2.6 (2.3-2.9)	Macek *et al.* (1969)
	0.6-1.5		7.2	6.1 (5.6-6.6)		1.7 (1.5-1.9)	Macek *et al.* (1969)
	0.6-1.5		12.7	3.2 (2.9-3.5)		1.5 (1.3-1.7)	Macek *et al.* (1969)
Oncorhynchus mykiss rainbow trout	-	-	-		1.2		F.W.P.C.A. (1968)
Oncorhynchus mykiss rainbow trout	5.5 cm	tech.96%/acetone	4			1.6 (1.4-1.9)	Sunderam *et al.* (1992)
			12			0.7 (0.5-0.9)	Sunderam *et al.* (1992)
Oncorhynchus mykiss rainbow trout	0.6	tech.	12			0.3 (0.3-0.4)	Nebeker *et al.* (1983)
	0.8	tech.	13			0.4 (0.3-0.5)	Nebeker *et al.* (1983)
	1.3	tech.	12			1.7 (0.8-3.7)	Nebeker *et al.* (1983)
	1.3	tech.	12			1.6 (1.4-1.9)	Nebeker *et al.* (1983)
Carassius auratus goldfish	-	35EC	-		1.4		N.R.C.C. (1975)
Cyprinus carpio carp	908-2724	EC	-	10			Mulla *et al.* (1967)
	5.0 cm	tech. 96%/acetone	25			0.1 (0.07-0.21)	Sunderam *et al.* (1992)
	20	tech.	26			1.98	Naidu *et al.* (1987)
Catostomus commersoni white suckers	0.9-2.5	tech./ethanol	10	8.1 (7.4-8.8)	6.4 (5.6-7.2)	3.5 -	Schoettger (1970)

Cont'd

Table 6-2 (Cont'd) Acute toxicity of endosulfan to freshwater fish.

Species	Size (g)	Formulation	Temperature (°C)	LC50 ($\mu g \cdot L^{-1}$) 24-h	48-h	96-h	Reference
Channa punctata snakehead murrel	juvenile (6-9 cm)	35EC/acetone	30			2.5 (1.9-3.2)	Devi *et al.* (1981)
	juvenile (6-9 cm)	tech.96%/acetone	30			4.8 (4.4-5.2)	Devi *et al.* (1981)
Channa punctata snakehead murrel	59.8	35EC/acetone	18			3.07 (2.43-3.87)	Haider and Inbaraj (1986)
	59.8	tech./acetone	18			5.78 (4.49-7.44)	Haider and Inbaraj (1986)
Sarotherodon mossambicus	5.9	35EC	27.6	10.4	6.7	4.3	Matthiessen and Logan (1984)
Labeo rohita carp	0.25	35EC/acetone	28			1.00 (0.98-1.02)	Rao *et al.* (1980)
	0.25	tech.96%/acetone	28			1.10 (1.07-1.12)	Rao *et al.* (1980)
Mystus vittatus catfish	6-10	35EC	26	0.32	0.26	0.24	Reddy and Gomathy (1977)
Mystus vittatus catfish	3.5	tech./acetone	28			2.2 (2.0-2.4)	Rao and Murty (1982)
Mystus vittatus catfish	6-10	35EC				0.67	Verma *et al.* (1980)
Mystus cavasius catfish	4.0	tech./acetone	28			1.9 (1.8-2.1)	Rao and Murty (1982)
Heteropneustes fossilis	21	tech./acetone	28			1.1 (0.9-1.3)	Rao and Murty (1982)
Heteropneustes fossilis	23.2	35EC/acetone	30			10.0	Singh and Narain (1982)
Heteropneustes fossilis	34.82	-	23	3.3 (2.9-3.5)	2.9 (2.6-3.2)	2.0 (1.8-2.2)	Singh and Srivastava (1984)
Tilapia mossambica	10	-	-			2.78	Rangaswamy and Naidu (1989)
Catla catla	0.3	35EC/acetone	28			1.05 (1.03-1.07)	Rao (1989)
Catla catla	0.3	tech.96%/acetone	28			1.84 (1.78-1.91)	Rao (1989)
Macrognathus aculeatum	13.2	tech.96%/acetone	28			3.5	Rao *et al.* (1981)
Lebistes reticulatus	-	35EC	-		1.4		N.R.C.C. (1975)

Cont'd

Table 6-2 (Cont'd) Acute toxicity of endosulfan to freshwater fish.

Species	Size (g)	Formulation	Temperature (°C)	LC50 ($\mu g \cdot L^{-1}$) 24-h	48-h	96-h	Reference
Pimephales promelas fathead minnow	-	23.5 EC	23.8	1.84 (1.65-2.05)	1.70	1.32 (1.13-1.54)	Kleiner *et al.* (1984)
	0.2	tech.	21			1.7 (1.5-2.3)	Nebeker *et al.* (1983)
	0.1	tech.	22			1.0 (0.8-1.3)	Nebeker *et al.* (1983)
	0.1	tech.	20			1.3 (0.7-2.4)	Nebeker *et al.* (1983)
	0.1	tech.	20			0.8 (0.8-0.9)	Nebeker *et al.* (1983)
Lepomis macrochirus bluegill sunfish Henderson (1966)				3.6			Pickering and
Brachydanio rerio zebra fish	0.18	tech.97%/acetone	25	1.6 (1.3-2.1)			Jonsson and Toledo (1993a)
Hyphessobrycon bifasciatus yellow tetra	0.73	tech.97%acetone	25	2.6 (2.3-2.9)			Jonsson and Toledo (1993a)
Rasbora heteromorpha harlequin fish	1.3-3.0 cm	tech.96%/acetone	20	0.09			Alabaster (1969)
Rasbora sp.	3.0 cm	tech.96%/acetone	25			0.2	Sunderam *et al.* (1992)
Gasterosteus aculeatus threespine stickleback	0.06	50WP	18-19	7.75 (1.6-16)	6 (1.6-16)		Ernst *et al.* (1991)
Anguilla anguilla european eel	20-30	tech.96%/acetone	22			41 (33-51)	Ferrando and Andreu-Moliner (1989)
Gambusia affinis mosquito fish	4.0 cm	tech.96%/acetone	25			2.3	Sunderam *et al.* (1992)
	2.0-2.5 cm	3EC	20			1.3 (1.14-1.43)	Naqvi and Hawkins (1988)
Oreochronius mossambicus	10	tech.94%/acetone	30	20	12	6	Ganesan *et al.* (1989)
Anabas testudineus	8	-	32	3.0 (2.5-3.6)	2.4 (2.1-2.7)	1.2 (1.1-1.3)	Rao and Murty (1980)

Cont'd

Table 6-2 (Cont'd) Acute toxicity of endosulfan to freshwater fish.

Species	Size (g)	Formulation	Temperature (°C)	LC50 (µg·L⁻¹) 24-h	48-h	96-h	Reference
Clarius batrachus catfish		tech.90%/ethanol	20	22.5 (19.0-61.0)	17.5 (17.0-18.0)	14.0 (13.4-14.5)	Gopal *et al.* (1981)
Clarius batrachus catfish	25-30	35EC		8.7 (7.5-10.0)	6.9 (5.6-7.5)	3.8 (3.2-4.4)	Bhatnagar *et al.* (1988)
Saccobranchus fossilis	5-10	35EC	18	8.1	7.7	6.6	Verma *et al.* (1982)
	5-10	35EC	18	11.1	10.9	10.8	Verma *et al.* (1982)
Melanotaenia duboulayi eastern rainbow fish	4.0 cm	tech.96%/acetone	25			2.5 (2.2-2.8)	Sunderam *et al.* (1992)
Bidyanus bidyanus silver perch	5.0 cm	tech.96%/acetone	25			2.3 (1.8-3.0)	Sunderam *et al.* (1992)
Macquaria ambigua golden perch	4.0 cm	tech.96%/acetone	25			0.5 (0.4-0.7)	Sunderam *et al.* (1992)
Nematolosa erebi bony bream	5.0 cm	tech.96%/acetone	26			0.2 (0.2-0.3)	Sunderam *et al.* (1992)

Table 6-3 Acute toxicity of endosulfan to different life stages of carp, *Cyprinus carpio* (adapted from Hashimoto *et al.* 1982).

Life Stage	Age [1] (days)	Weight (g)	24-h LC50 ($\mu g \cdot L^{-1}$)
eyed egg	-	-	2,500
sac fry	1-3	-	560
floating fry	5-6	-	410
1-week-old fry	9-10	0.013	11
2-week-old fry	17-19	0.038	1.6
4-week-old fry	25-33	0.23	1.5
8-week-old fry	50-60	0.87	2.0
11-week-old fry	70-80	2.34	4.9

Age [1] - number of days after floating or feeding

Table 6-4 Acute toxicity of technical grade endosulfan and its isomers α and β to fish.

Species	96-h LC50 (μg L⁻¹) α-endosulfan	Technical	ß-endosulfan	Reference
Catla catla	0.36 (0.35-0.37)	1.84 (1.78-1.91)	7.67 (7.52-7.82)	Rao (1989)
Labeo rohita	0.33 (0.32-0.34)	1.10 (1.07-1.12)	7.10 (7.02-7.23)	Rao *et al.* (1980)
Channa punctata	0.16 (0.11-0.21)	4.8 (4.4-5.2)	6.6 (6.6-6.7)	Devi *et al.* (1981)

Table 6-5 Acute toxicity of different formulations of endosulfan to fish.

Species	96-h LC50 ($\mu g \cdot L^{-1}$) 35EC[1]	Technical (96%)[2]	4DU[3]	Reference
Catla catla	1.05 (1.03-1.07)	1.84 (1.78-1.91)	2.20 (2.15-2.26)	Rao (1989)
Labeo rohita	1.00 (0.98-1.02)	1.10 (1.07-1.12)	1.25 (1.21-1.30)	Rao *et al.* (1980)
Channa punctata	2.5 (1.9-3.2)	4.8 (4.4-5.2)	16 (15-17)	Devi *et al.* (1981)
Channa punctata	3.07 (2.43-3.87)	5.78 (4.49-7.44)	-	Haider and Inbaraj (1986)

[1] 35EC - emulsifiable concentrate - 35% active ingredient

[2] Technical (96%) - 96% pure endosulfan

[3] 4DU - dust - 4% active ingredient

Table 6-6 Effect of temperature on the acute toxicity of endosulfan to freshwater fish.

Species	Size (g)	Temperature (°C)	96-h LC50 ($\mu g \cdot L^{-1}$)	Reference
Oncorhynchus mykiss rainbow trout	1.0-1.8	1.5	0.8	Schoettger (1970)
	1.0-1.8	10.0	0.3	
Oncorhynchus mykiss rainbow trout	0.6-1.5	1.6	2.6	Macek *et al.* (1969)
		12.7	1.5	
Oncorhynchus mykiss rainbow trout	5.5 cm	4	1.6	Sunderam *et al.* (1992)
		12	0.7	
Catostomus commersoni white sucker	0.9-2.5	10	3.5	Schoettger (1970)
	0.9-2.5	19	3.0	
Anguilla anguilla european eel	20-30	22	42	Ferrando *et al.* (1987)
	20-30	29	20	
Heteropneustes fossilis	11.1	18	12.7	Singh and Narain (1982)
	11.1	30	7.7	
	43.8	18	22.8	Singh and Narain (1982)
	43.8	30	15.1	

Table 6-7 Acute toxicity of endosulfan and its metabolites to fish (adapted from Knauf and Schulze 1973).

Species	48-h LC50 ($\mu g \cdot L^{-1}$)					
α-hydroxyether	35 EC	endosulfan sulfate	endosulfan lactone	endosulfan alcohol	endosulfan ether	endosulfan
Lebistes reticulatus 900 guppy	4.0	6.0	25,000	7,500	2,500	
Carassius auratus goldfish	8.0	75	5,000	7,500	3,500	
Idus melanotus	8.0	12.5	7,500	5,000	-	

Table 6-8 Endosulfan isomers and metabolites found in fish tissue following 96-h exposures.

Species	Endosulfan Isomers and Metabolites							Reference
	Isomer A	Isomer B	Sulfate	Lactone	Alcohol	Ether	α-hydroxy-ether	
Catla catla								
Liver	+[1]	+	+	+	+	++[2]	ND[3]	Rao (1989)
Kidney	+[4]	+	+	+	+	ND	ND	
Gallbladder	-	-	-	-	-	-	-	
Anterior Gut[5]	-	-	-	-	-	-	-	
Posterior Gut[6]	-	-	-	-	-	-	-	
Whole Gut	-	-	-	-	-	-	-	
Brain	-	-	-	-	-	-	-	
Gills	-	-	-	-	-	-	-	
Muscle	+	+	ND	ND	ND	ND	ND	
Channa punctata								
Liver	+	+	ND	+	+	++	ND	Devi *et al.* (1981)
Kidney	+	+	ND	+	+	++	+	
Gallbladder	-	-	-	-	-	-	-	
Anterior Gut	-	-	-	-	-	-	-	
Posterior Gut	-	-	-	-	-	-	-	
Whole Gut	-	-	-	-	-	-	-	
Brain	+	+	ND	+	+	ND	+	
Gills	+	+	ND	+	+	ND	+	
Muscle	-	-	-	-	-	-	-	
Labeo rohita								
Liver	ND	+	ND	+	+	++	+	Rao *et al.* (1980)
Kidney	ND	+	+	ND	+	+	+	
Gallbladder	-	-	-	-	-	-	-	
Anterior Gut		+				N	ND	
Posterior Gut	ND	ND	ND	+	+	+	+	
Whole Gut	-	-	-	-	-	-	-	
Brain	ND	ND	ND	ND	ND	ND	ND	
Gills	+	+	+	ND	+	+	+	
Muscle	+	+	+	ND	ND	+	+	

Table 6-8 (cont'd) Endosulfan isomers and metabolites found in fish tissue following 96-h exposures.

Species	Endosulfan Isomers and Metabolites							Reference
	Isomer A	Isomer B	Sulfate	Lactone	Alcohol	Ether	α-hydroxy-ether	
Macrognathus aculeatum								
Liver	+	+	++	+	+	++	ND	Rao *et al.* (1981)
Kidney	+	+	++	+	+	++	ND	
Gallbladder	+	+	++	+	+	++	ND	
Anterior Gut	-	-	-	-	-	-	-	
Posterior Gut	-	-	-	-	-	-	-	
Whole Gut	+	+	++	+	ND	ND	ND	
Brain	+	+	++	+	ND	ND	ND	
Gills	+	+	++	-	-	-	-	
Muscle	+	+	++	+	ND	ND	ND	
Heteropneustes fossilis								
Liver	+	+	++	ND	++	ND	ND	Rao and Murty (1982)
Kidney	+	+	++	ND	ND	ND	ND	
Gallbladder	-	-	-	-	-	-	-	
Anterior Gut	-	-	-	-	-	-	-	
Posterior Gut	-	-	-	-	-	-	-	
Whole Gut	+	+	++	ND	ND	ND	ND	
Brain	+	+	++	ND	ND	ND	ND	
Gills	+	+	++	ND	ND	ND	ND	
Muscle	+	+	++	ND	ND	ND	ND	
Mystus cavasius								
Liver	+	+	+	+	ND	+	+	Rao and Murty (1982)
Kidney	+	+	+	+	+	ND	+	
Gallbladder	-	-	-	-	-	-	-	
Anterior Gut	-	-	-	-	-	-	-	
Posterior Gut	-	-	-	-	-	-	-	
Whole Gut	+	+	+	ND	ND	ND	ND	
Brain	+	+	+	ND	ND	ND	ND	
Gills	+	+	+	ND	ND	ND	ND	
Muscle	+	+	+	ND	ND	ND	ND	

Table 6-8 (cont'd) Endosulfan isomers and metabolites found in fish tissue following 96-h exposures.

Species	Endosulfan Isomers and Metabolites							Reference
	Isomer A	Isomer B	Sulfate	Lactone	Alcohol	Ether	α-hydroxy-ether	
Mystus vittatus								
Liver	ND	+	++	ND	+	+	ND	Rao and Murty (1982)
Kidney	+	+	ND	ND	ND	ND	ND	
Gallbladder	-	-	-	-	-	-	-	
Anterior Gut	+	+	++	ND	ND	ND	ND	
Posterior Gut	-	-	-	-	-	-	-	
Whole Gut	-	-	-	-	-	-	-	
Brain	+	+	++	ND	ND	ND	ND	
Gills	+	+	++	ND	ND	ND	ND	
Muscle	+	+	++	ND	ND	ND	ND	
Anabas testudineus								
Liver	+	+	+	+	+	++	+	Rao and Murty (1980)
Kidney	ND	ND	+	ND	+	++	ND	
Gallbladder	ND	ND	+	ND	+	++	ND	
Anterior Gut	-	-	-	-	-	-	-	
Posterior Gut	-	-	-	-	-	-	-	
Whole Gut	ND	ND	+	ND	+	++	ND	
Brain	ND	ND	ND	ND	ND	ND	ND	
Gills	+	+	+	ND	ND	ND	ND	
Muscle	+	+	ND	ND	ND	ND	ND	

1 + isomer/metabolite present
2 ++ principal degradation product
3 ND not detected
4 - not analyzed
5 anterior gut - anterior to the entrance of the bile duct
6 posterior gut - posterior to the entrance of the bile duct

Table 6-9 Lipid content and distribution of endosulfan in tissues of *Sarotherodon macrochir* three weeks after termination of endosulfan spraying (6 separate spray events) for tsetse fly control in Botswana, Africa (adapted from Matthiessen *et al.* (1982).

Tissue	% Lipid	Endosulfan Residue in Tissue[1] ($\mu g \cdot kg^{-1}$ wet wt.)
Visceral Adipose	42.1	1600
Gill	6.8	180
Ovary	6.4	390
Bile	4.8	360
Liver	4.2	190
Gut	1.9	<100
Caudal Muscle	0.8	<100

[1]each value represents a pooled sample of 7 adult fish

Table 6-10 Endosulfan residues and lipid content of liver for fish collected from rivers in an agricultural area in Australia (adapted from Nowak and Julli 1991).

Species	Summer 1988		Winter 1988		Summer 1989	
	Endosulfan Residue ($\mu g \cdot kg^{-1}$ wet wt.)	Lipid Content (%)	Endosulfan Residue ($\mu g \cdot kg^{-1}$ wet wt.)	Lipid Content (%)	Endosulfan Residue ($\mu g \cdot kg^{-1}$ wet wt.)	Lipid Content (%)
Tandanus tandanus catfish	205	3.4	6	2.5	23	2.5
Cyprinus carpio common carp	463	10.6	4	4.2	33	6.2
Nematolosa erebi bony bream	912	25.9	30	14.4	64	16.2

Table 7-1 Acute toxicity (LD50s) of endosulfan to avian species.

Species	Sex	Age (months)	LD50 ($mg \cdot kg^{-1}$)	95% Confidence Limit ($mg \cdot kg^{-1}$)	Reference
Mallard	M&F	36 hours	27.8	22.8-33.8	Hudson *et al.* (1972)
	M&F	7 days	6.47	5.19-8.05	Hudson *et al.* (1972)
	M&F	1	7.89	5.77-10.8	Hudson *et al.* (1972)
	M	3	33.0	23.8-45.8	Hudson *et al.* (1984)
	M&F	6	34.4	26.1-45.3	Hudson *et al.* (1972)
	M	12	45.0	36.2-56.0	Hudson *et al.* (1984)
	F	12	31.2	20.8-46.6	Hudson *et al.* (1984)
	?	?	205-243	?	Gulf South Research Inst. (1972, cited in NRCC 1975)
Ring-necked Pheasant	M	3-4	80-160	-	Hudson *et al.* (1984)
	F	3-4	190	137-263	Hudson *et al.* (1984)
	F	12	>320	-	Hudson *et al.* (1984)
	?	?	620-1000	-	Worthing and Hance (1991)
Bobwhite Quail	?	?	50-56	?	Gulf South Research Inst. (1972, cited in NRCC 1975)
Japanese Quail	?	?	85-106	?	Gulf South Research Inst. (1972, cited in NRCC 1975)
Starling	?	?	35	11-110	Schafer (1972)

NOTE: Hudson *et al.* (1984) results are 14-day LD50s. Schafer (1972) reports 7-day LD50s. Technical grade endosulfan used in all tests rather than commercial formulation.

Table 7-2 Dietary toxicity (LC50s) of endosulfan for avian species.

Species	Age (days)	LC50 ($mg \cdot kg^{-1}$ feed)	95% Confidence Limit ($mg \cdot kg^{-1}$ feed)	Reference
Mallard	16	1,053	781-1,540	Hill *et al.* (1975)
Ring-necked Pheasant	10	1,275	1,098-1,482	Hill *et al.* (1975)
Bobwhite Quail	9	805	690-939	Hill *et al.* (1975)
Japanese Quail	14	≈1,250	-	Hill *et al.* (1975)

NOTE: Hill *et al.* (1975) results are based on 8-day LC50s, 5 days on treated feed followed by 3 days on untreated feed. Technical grade endosulfan used rather than commercial formulation.

TABLE . 3 Acute Toxicity of Endosulfan to Amphibians.

Species	Life Stage	Test Duration (hours	LD50 (mg/L)	LD100 (mg/L)	95% Confidence Limit (mg/L)	Reference
Rana tigrina	tadpole	96	0.0018		0.0014 0.0022	Gopal *et al.* 1981
	tadpole	96	0.004			Abbasi and Soni 1991
	tadpole	144	0.0007			Abbasi and Soni 1991
Bufo bufo		24		0.015	--	Oeser *et al.* 1971
	tadpole	48		0.015	--	Lüdemann and Neumann 1960, 1962
Bufo bufo japonicus	tadpole	48	9.0			Hashimoto and Nishiuchi 1981
Bufo meianostictus		96	0.1230		0.1184 0.1278	Vardia *et al.* 1984