CHAPTER 5

Ecological Impacts of Major Forest-Use Pesticides

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Abstract: Assessing the potential for ecological impacts of pesticides requires a hierarchical approach with research ranging from simple laboratory to complex field experiments and operational monitoring. While all levels of study provide useful information, higher tier research has inherently greater environmental relevance and inference potential. In this chapter, selected higher tier studies relating to the use of herbicides glyphosate and triclopyr, as well as the insecticides *Bacillus thuringiensis* var. *kurstaki* (Btk) and diflubenzuron in the forest sector are reviewed. These case examples illustrate scenarios in which higher tier studies either negate or support the presumptions of risk derived from results of lower tier experiments. Specifically, assessment of the cases for glyphosate and Btk support their continued judicious use as environmentally acceptable components of integrated vegetation and insect pest management strategies. In contrast, higher level studies confirm risk postulates associated with typical forest-sector use patterns for triclopyr ester and diflubenzuron. Mitigation measures are required to ensure that use of these latter compounds do not pose undue risk to sensitive non-target organisms. In a broader context, the ecological implications of pesticide use in the forest sector must be considered in light of the fact that any management action, including the "no intervention" option, carries both economic and ecological risk. Strict adherence to the weight of scientific evidence principle, incorporation of knowledge gained from all levels of investigation, and a balanced assessment of relative risks of all potential options are considered primary requisites of comprehensive risk analysis and effective decision making.

INTRODUCTION

Truhaut [1] described ecotoxicology as that branch of the discipline concerned with the toxic effects of natural or synthetic pollutants on the constituents of ecosystems. As noted by Butler [2], this concept carries the inherent requirement to consider how the toxicant is released, its potential transformation and its possible transport to other compartments, since these are the primary determinants of exposure and effect. Potential effects must be considered at multiple scales, including those of biological organization (organism, population, or community), space (local to landscape) and time (days to years). These concepts are particularly relevant to the assessment of ecological impacts of pesticides in the forest sector where they may be applied to assist in regeneration or protection of forest stands and where there is potential exposure of a diverse array of organisms within highly interconnected ecosystem compartments.

Ecotoxicological risks associated with modern forest-use pesticides are quite unlike those of historic compounds such as DDT. However, the potential for both direct and indirect effects exists, and such risks are often the dominant element of public concern and policies associated with this forest management practice, as well as with forest certification schemes. Forest pesticide use varies across the globe, principally in relation to the size and accessibility of the resource, primary crop species and the value of commodities derived there from. In some countries blessed with huge areas of natural forests (e.g. Canada, Russia, USA), pesticides are applied to only a very small proportion of the forest land base that is managed for commercial production of high value products such as sawn wood, panels or pulp and paper. In other countries (e.g. New Zealand, Australia, Finland, Sweden and south-eastern USA) relatively more intensive "plantation" management may be employed for the same general purpose and of course gradients of relative management intensity occur in most countries.

While the focus of this chapter is on ecotoxicological risks of forest-use pesticides, such risks must be considered within the broader context of assessing both risk and benefit of this or alternative forest management actions. Few, if any forest managers would choose to apply pesticides if there were not substantial benefits associated with such treatments. For example, herbicides are recognized as the most effective tool for controlling competing vegetation to favour partitioning of essential light, water, nutrients and growing space to the desired crop species rather than to weedy competitors [3]. Wagner *et al.* [4] recently reviewed results from 60 of the longest-term studies in Canada, the USA, South Africa, Brazil, New Zealand and Australia, documenting that the majority of studies show 30 to

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500% increases in wood volume as well as reduced rotation periods from effective vegetation control treatments. Positive outcomes are reflected in significantly enhanced regeneration success and overall sustainable management of forest resources. A diverse array of insect pest species are capable of causing significant economic or ecological damage in major plantations or natural forest stands [5, 6]. Both chemical and biological insecticides are applied to protect semi-mature or mature high value forest stands or to slow the spread of invasive species across the landscape and thus mitigate either economic or ecological losses. In cases where no effective chemical or biological controls are applied, devastating losses are typically the result. For example in Canada, no effective pesticides have been developed or applied to control the epidemic outbreak of mountain pine beetle in lodgepole pine stands. This single insect pest now affects a forest area in excess of 14.5 million ha in the province of British Columbia [7] an area essentially equivalent to that of England. As the beetle moves across the Rocky Mountain divide into Alberta it threatens stands of other pine species including jack pine that spans the boreal forest region across country with massive implications in terms of economic loss, carbon release to the environment and unknown ecological effects in a region not previously adapted to this pest species.

Pesticide risk should also be considered in relation to specific use patterns and proportional use. In comparison to agriculture, pesticide use in forestry involves substantially fewer active ingredients as well as dramatically lower use frequency and proportion of the total productive land area treated in any given year. For example, pesticide use in Canadian forestry accounts for only ~2% of total pest control products sold in that country. Only two active ingredients, the herbicide glyphosate and the microbial insecticide *Bacillus thuringiensis* var. *kurstaki* (Btk) have any significant degree of use, each comprising more than 90% of the total forest area treated with a herbicide or insecticide respectively, a determination based on 2007 statistics for pest control product use in that sector [8]. Similarly, pesticide use in plantation forestry in Australia accounts for only 0.7% of the total annual national expenditures on pesticides [9]. The latter report presents detailed analysis of pesticide expenditures in agricultural crops as compared to forestry. Results emphasize the dramatically higher use frequency and hence expenditures associated with pesticide use in agricultural crop production. To a large degree, this reflects the common practice of multiple pesticide applications on an annual basis to much of the agriculture land base. In contrast, individual forest stands rarely, if ever receive annual pesticide treatments and frequency of use is typically quite low. Even under intensive forest management regimes, the total number of pesticide applications during a rotation period is unlikely to exceed four; that is two herbicide treatments in the early regeneration phase and two insecticide treatments when trees are semi-mature to mature. However, rotation periods vary markedly with forest crop species ranging from as little as 8 to 10 years for example in short rotation eucalypt plantations of Australia, to 80 years or more for spruce stands in the boreal forests of Canada. The total proportion of the productive forest land base treated is also an important consideration in ecotoxicological risk assessments. Again, on a comparative basis, agricultural food crop production often involves essentially 100% of the land base receiving at least one pesticide treatment each year, whereas production of fibre typically involves pesticide application to only a very small proportion of the commercial forest land base annually. However, exceptional cases have been documented historically, including for example massive spruce budworm outbreak in New Brunswick, Canada where almost 4 million ha of forest land was treated with insecticides in one year [5]. While these statistics vary with jurisdiction, year and pesticide type, the point is well exemplified by herbicide use in Canadian forestry where <1% of the commercial forest land base is treated in any given year [10].

Considered in combination, and particularly in relation to agricultural pesticide use, the few active ingredients employed in forest management, their relatively low use frequency, the minor proportion of total forest land area treated and the resultant lower environmental loadings (*i.e.* mass of total pesticide applied per unit area), public concern over pesticide use in forestry seems disproportionately high. For example a poll of 2500 Canadians indicated 71% opposed the use of chemicals in the forest [11]. As noted by Guynn *et al.* [12], public perception of risks may contrast significantly with scientific conclusions based on the weight of scientific evidence from the cumulative primary literature. However, under current socio-political systems in most countries, public opinion carries significant influence over decision making and management policy, thus controlling the “social license to operate” on publicly owned lands. Current examples include restrictions or outright bans on chemical pesticide use in the forest-sector in certain political jurisdictions of Canada and the USA, despite registration and approval for these specific uses by federal regulatory agencies. Another example is the mandatory requirement to reduce or eliminate the use of chemical pesticides as a forest management option in some forest certification schemes [13], presumably reflecting the wishes of a more environmentally conscious and engaged consumer base.

Scope Statement

Ecological risk estimation is generally considered as a tiered or hierarchical process which requires fundamental knowledge and data derived from scientific disciplines of environmental chemistry, biology, ecology and toxicology [14]. Production of these primary data is a legislative requirement of government regulatory bodies in many countries (e.g. the United States Environmental Protection Agency, the Canadian Pest Management Regulatory Agency and the Australian Pesticides and Veterinary Medicines Authority). Each of these regulatory agencies, as well as many other regional regulatory agencies, conduct independent reviews of the data prior to national registration and specific regional or sectoral use of pesticides. General discussion of the fundamental environmental fate and toxicology data requirements are discussed in chapters 1 and 2 of this text and will not be considered in detail here. Readers interested in more specific details on these fundamental toxicological data are directed to the Pesticide Information Profile briefs available on the EXTOXNET website [15], which provides convenient summaries for each pesticide. The United States Department of Agriculture – Forest Service documents also available *via* the internet [16] are another comprehensive source of data and information on how such data may be used directly in human health and environmental risk analysis.

Over and above these fundamental regulatory data requirements, numerous higher tier experiments and field investigations are conducted to inform the process. Among the various classes of pesticides that might be applied in forest management, herbicide and insecticide use predominates, with relatively minor amounts of fungicides being broadcast applied to plantations or natural forest stands [6]. As such, discussion in this chapter will be restricted to herbicidal and insecticidal compounds and based on four selected case examples (two for each pesticide class). Case examples were chosen as representative compounds most commonly used in the forest sector, or because they emphasize key ecotoxicological issues which are integral to the continuous debate over pesticide use both in the forest sector and more generally. The examples put forward in this chapter are intended to demonstrate the wealth of scientific information pertinent to possible ecological impacts of major forest-use pesticides and to emphasize the importance of higher tier manipulative field experiments and monitoring as critical components of the overall risk assessment process governing their regulation and use.

USE PATTERNS AND EXPOSURE ASSESSMENT FOR MAJOR FOREST-USE PESTICIDES

The critical determinant of any toxicological effect is the dose; that is the level of the toxicant which occurs at the physiological site of activity within the organism. As such, toxicological effects are often directly proportional to environmental exposure concentrations with due consideration for modulating effects associated with the fundamental biology or behaviour of the receiving organism. For example, the feeding rate and preferences of different insects may influence exposure, while seasonal development of plant cuticles may act as a barrier to herbicide uptake in plants. In the case of forest-use pesticides, which are intentionally applied to known areas for very specific purposes, typical use patterns and application rates (Table **1**) are, in turn, the key determinants of potential environmental exposures. The actual application rate employed is selected by experienced forest managers based on the degree of infestation, susceptibility of the pest problem and cost considerations. Often the rates employed operationally may be less than the maximum allowed.

As competing vegetation and insect pest problems in the forest sector often occur at very large spatial scales and with substantial infestation intensities, broadcast techniques are often the only practically feasible method for applying the chemical to target sites. Forest-use herbicides, excepting soil active compounds, are applied with the specific intent of impinging the maximum possible mass of active ingredient on foliage of the competing vegetation canopy. Similarly, insecticides are typically applied such that they impinge predominantly within the crop tree canopy upon which many insect pests feed. Thus, non-target organisms residing or foraging in targeted plant canopies have the greatest likelihood of direct exposure [17, 18]. However, since not all of the depositing spray cloud is impinged within the target canopy, exposures of ground dwelling, soil or aquatic organisms may occur to some extent through either direct or indirect mechanisms (e.g. by rain-wash) and cannot be completely disregarded. Such exposures may be of particular importance in cases where highly sensitive or rare species are known to occur. The development and use of various new technologies including low drift nozzles, electronic guidance systems on spray aircraft and geographic information system mapping of spray blocks have greatly improved control and optimization of spray deposition. When used in conjunction with recently developed decision support systems such as SprayAdvisor, such advanced tools and techniques can substantially reduce the probability of depositing toxicologically significant levels of pesticide outside the targeted spray area[10].

Table 1: Comparative examples of maximal and typical use rates, as well as calculated and actual environmental concentrations observed in field research and monitoring studies for four major forest-use pesticides.

Active Ingredient	**Country**	**Registered end-use product examples**	**Typical use pattern**	**Use rates in forestry***	
				Label Max.	Typical
Glyphosate	Canada USA	Vision VisionMax Accord SP Roundup Original	Aerial -Conifer Release	2.14 2.16 11.2; 4.2	1.9[8,81] 2.7[15] 2.63[37]
Triclopyr butoxyethyl ester	Canada USA	Release® Garlon 4	Ground: foliar & woody weed control	3.84	2.3[15]
Bacillus thuringiensis var. *kurstaki* (Btk)	Canada USA	Foray 76 Foray 48B Dipel 8L	Aerial broadcast – Spruce and Jackpine budworms, Gypsy moth, Douglas Fir Tussock Moth, Spruce budworm, Painted Apple Moth	60 BIU/ha 60 BIU/ha	30-60 BIU/ha[8] 49-99 BIU/ha[18]
Diflubenzuron	USA	Dimlin 4L Dimlin 25W	Aerial – gypsy moth	0.07 0.035	0.009-0.070[125] 0.009-0.035[125]

Superscripted numbers in brackets correlate directly to references from which data were obtained.

* kg/ha unless otherwise noted

Accord®, Roundup Original®,Vision® and VisionMax® are registered products of the Monsanto Co., St. Louis, Missouri; Garlon 4®, and Release® are registered products of DowAgroSciences, Indianapolis IN; Foray 76®, Foray 48B® and Dipel® are registered products of Valent Biosciences, Toronto ON; Dimlin 4L® and Dimlin 25W® are registered products of Uniroyal Chemical Co., Bethany CT;

Table 2: Observed concentrations, primary mechanisms of degradation or dissipation and persistence estimates for major use pesticides in environmental compartments of various forest ecosystems.

Active Ingredient	**Environmental Compartment**	**Maximum Conc. (mg/L or ppm)**	**Primary Mechanisms of Degradation or Dissipation**	**DT50 (days)**
Glyphosate	Vegetation Litter Soil Water	529[59] 322[43], 8.3[60] 1.4[43], 1.5[60] 550[71]	Uptake & translocation Microbial Microbial Microbial, sorption	2[59] 12[60] 10[60] 4.2 to 26.4[70]
Triclopyr ester or acid	Target Vegetation Litter Soil Water	1630[59], <450[39], 127[35] 53[35] 0.73[35], 45.7[60] 0.35[76	Uptake & translocation Photolysis Microbial Microbial Based-catalyzed hydrolysis, photolysis	4[59], 31-202[35] 31[35], 39[60] 60[60], 14[84] 4-8[93]
Bacillus thuringiensis var. *kurstaki* (Btk)	Vegetation Litter Soil Water	480[18] n/a n/a n/a	UV kill of endospores, alkaline or enzymatic hydrolysis of endotoxins[19]	1-64[20] 100-200[92] >70[21]
Diflubenzuron	Target Vegetation Litter Soil Water	n/a n/a n/a 0.006-0.014[112]	Photolysis Microbial Microbial Photolysis, hydrolysis, sorption	<21[22] n/a 2.1[117], 8.6[117] <14[23], 3-8[113]

Superscripted numbers in brackets correlate directly to references from which data were obtained.

Pesticides currently in widespread use in the forest sector may be generally characterized as non-persistent, susceptible to microbial degradation, photolysis, hydrolysis or other degradation mechanisms and non-bioaccumulatory. Extensive scientific knowledge on their fundamental physico-chemical properties and on their environmental fate under both laboratory and representative field scenarios exist. From field experiments under quasi-operational conditions, empirical estimates of initial concentrations observed in various environmental compartments as well as time to 50% dissipation (DT50 or half-life) estimates are also available as shown in Table **2**.

DT50 values provided in Table **2** indicate that residues of pesticides commonly used in the forest sector are relatively short-lived in all major environmental compartments. As such, exposure regimes are typically characterized by peak concentrations occurring shortly after application and with diminishing magnitude of exposure through time. The duration of exposures are often curtailed by the combined effect of environmental degradation and dissipation mechanisms which are active in these compartments. The resultant changes to chemical structure or bioavailability may significantly modulate exposure regimes and thus potential toxicological effect. Commonly, where wildlife exposures to pesticides occur, the exposure regime may be characterized as a pulse exposure of relatively short duration. In some cases, natural environmental exposure regimes differ markedly from those typically employed in standard tier 1 toxicity testing protocols in which test concentrations are artificially maintained at some constant high level. Considering all of the foregoing information, Tier 1 hazard quotient analyses, which are based on estimated exposure under the assumption of maximum labeled use rates and effect endpoints derived from atypical exposure regimes, should be considered as worst case risk estimates. Often the magnitude and duration of real world exposures, as well as toxicity observed in studies conducted *in situ*, are substantially lower than those predicted from simple hazard quotient analyses. Nonetheless, the majority of these types of risk analyses demonstrate that major forest-use pesticides do not pose substantial risks of direct toxicity to most wildlife species. Risks are generally greater in cases where the mechanism of activity is common to target and non-target organisms alike (e.g. acetylcholine esterase inhibition) and where both groups may be equivalently exposed (e.g. target and non-target insects in forest canopies) or where a particular group of organisms are uniquely sensitive to the pesticide or constituents of the pesticide formulation. While it is recognized that there are exceptions to most, if not all, generalities (some of which are described below), ecological impacts associated with the modern pesticides currently in widespread use for forest insect pest control and vegetation management are much more likely to occur through indirect mechanisms, such as changes in habitat or food availability, as opposed to direct acute toxicity.

FOREST-USE HERBICIDES

Among countries leading international trade in forest-resource based products, only a handful of herbicidal active ingredients are registered and commonly used to control competing vegetation as a means of enhancing forest regeneration (Table **1**). Given that vegetative competition is most critical during the early establishment phase of forest regeneration [24], herbicide applications are typically made to prepare the site just prior to planting or in the very early stages (1-3 years) subsequent thereto. It is important to recognize that herbicide treatments therefore follow shortly after the major physical disturbances which result from harvesting and planting operations. In ecological terms, this is a transient stage in the cycle characterized by dynamic change and relatively rapid vegetative succession. Immediately following the physical disturbance of harvesting, sites typically become dominated by pioneer plant species well adapted to the high light intensities, disturbed soils and fluctuating temperatures which are often characteristic. As such, the potential changes in ecological structure and function that may be induced by herbicide treatments, must be considered in the context of the typical ecological dynamics of the sites to which they are applied and with due consideration to the dynamics in the broader forest landscape to which that specific site is connected [25-27].

The environmental fate and effects of herbicides used in forest vegetation management have been extensively investigated at experimental scales ranging from small laboratory studies to whole ecosystem manipulations. Several directly relevant reviews have been published previously [28-37, 52]. Independent regulatory reviews conducted in several countries (e.g. USA, Canada and Australia) with significant herbicide use in the forest sector consistently conclude that when applied in accordance with their specific product labels, such uses do not pose a substantive risk to wildlife or general environmental health. A special issue of the Wildlife Society Bulletin considers the transport and direct toxicity of many of many herbicides noted here [29]. A discussion of indirect influences of herbicide products used predominantly in the south-eastern USA on forest biodiversity [30] and wildlife habitat [12] is also included in the same publication. Collectively, the authors drew the following general conclusions from their review of the pertinent scientific literature:

- Herbicides most commonly used for vegetation management in forestry (glyphosate, triclopyr, imazapyr, sulfometuron, metsulfuron methyl, hexazinone) degrade quickly once they enter the environment and thus are neither persistent nor bioaccumulative.
- As modern herbicides have been designed to target biochemical processes unique to plants, they exhibit a low level of direct toxicity to animals.
- When used according to label instructions, modern silvicultural herbicides pose little risk to wildlife.
- Due to the high resilience of floral communities, plant species richness and diversity rebound rapidly after single herbicide treatments, with short- and long-term compositional shifts according to the selectivity and efficacy of the herbicide used.
- Under more intensive management regimes including multiple applications of herbicides, the shortened period of suitable habitat and reduction in habitat quality may reduce populations of disturbance-dependent species, however, the scale of application and the landscape context will determine the level of effects on local or regional populations.
- Detailed studies on influences of silvicultural treatments, including herbicides, on amphibian and reptile communities are especially needed.
- Despite these findings, public opinion against forest herbicides often has limited or restricted their use, likely due to common public values associated with forests and a lack of technical knowledge.

These conclusions are drawn largely from studies conducted in the south-eastern USA. However, they are further supported by results derived from several higher-tier studies conducted in Canada and in other major forest regions and may thus be considered as generally applicable. Below, case study examples for two different herbicides (glyphosate and triclopyr) are presented to illustrate scenarios in which ecotoxicological field studies demonstrated substantially differing levels of risk and the value of conducting detailed studies under real-world conditions as a critical component of a hierarchical approach to ecotoxicological risk assessment.

Glyphosate

As well as being the dominant herbicide in modern agriculture [31], glyphosate is also one of the most widely used herbicides in the forest sector around the globe including in Canada, the USA and Australia. For example, glyphosate-based products have accounted continuously for more than 93% of the total forest market in Canada for the 15 year period from 1992 through 2006 [8]. The knowledge base pertaining to the ecotoxicology of glyphosate is arguably the most extensive ever developed for a forest-use herbicide. The general environmental behaviour and toxicology of this herbicide has been the subject of several major independent reviews [32-35]. In addition, a seminal text presents much of the historical background and detailed information on all aspects of this unique compound in the early years post-discovery [36]. A search of several electronic databases provided several hundred records of primary scientific literature specific to the fate and effects of glyphosate in forest ecosystems. Many of these are field studies involving formulated end-use products applied at typical or maximal application rates and designed to examine the fate and effects under natural conditions typical of major forest uses. In addition, a number of other field studies are currently being conducted to address specific issues of scientific, public or operational forestry interests.

Since its discovery and introduction by Monsanto, numerous formulations of glyphosate have been registered and used in forest vegetation management globally. More recently, with the loss of patent control, multiple manufacturers are generating “generic” glyphosate products and more than 35 different formulations are used in the USA alone [37]. From both a use and ecotoxicological perspective, not all formulations are equivalent, largely owing to differences in the exact chemical composition of the products but also because of differences in application methods and rates as specified on the product labels. Several formulations contain different glyphosate salts or different surfactant blends which may significantly influence the uptake of the chemical in plants or potential ecotoxicological effects. However, in general, it is known that glyphosate is rapidly taken up by the plant following application of the formulated product and thereafter translocated to active growing tissues in both the aerial and root structures. As such, it is particularly effective for control of biennial or perennial species which self-propagate from basal sprouts, roots or rhizomes. Plants with this type of reproductive strategy are often the most problematic in forestry, particularly because they tend to be very poorly controlled by mechanical techniques. Often mechanical cutting actually stimulates more extensive growth, thereby exacerbating rather than alleviating competition with more desirable crop species. The mechanism of action for glyphosate involves blockage of a specific enzyme (5-enolpyruvyl-shikimate-3-phosphate synthetase or EPSPS) in the synthesis of aromatic amino acids. This biosynthetic pathway exists in both plants and microorganisms but not in higher animals [38, 39]. Owing to its highly plant-specific mode of action, direct effects of glyphosate on animals generally require much higher dose levels than would be typically encountered in natural environments, thus conferring a substantial level of safety for many wildlife species that may be potentially exposed. The environmental fate and persistence of glyphosate has been examined in vegetation, litter, soil, and water compartments of forest ecosystems ranging from the Pacific coastal forests in both the USA [40, 41] and in Canada [42, 43], to high latitude coastal and interior forest sites in Alaska [44], in southern and northern deciduous forests of the USA [45], in boreal forest sites of central Canada [46-48] and in the Acadian forest region of eastern Canada [49, 50]. The results of these extensive field studies allow for broad inferences on the environmental fate of glyphosate in forest ecosystems. In general, it is known that glyphosate is effectively impinged within the target canopy, with relatively low residues in ground vegetation or in soils. In all compartments, glyphosate is susceptible to rapid microbial degradation and thus non-persistent. It binds strongly to essentially any organic substrate including organic matter and clay particles of sediments and soils, and thus shows essentially no tendency to leach or move laterally with surface runoff even though it has relatively high solubility in water. The time to 50% dissipation for glyphosate in these various environmental compartments is provided in Table **2**. The primary degradation product is aminomethylphosphonic acid (AMPA) and several studies indicate that AMPA is also non-persistent under typical forest environmental conditions. At least one assessment [51] has focused specifically on AMPA which suggests that it provides little risk to aquatic organisms.

The United States Department of Agriculture – Forest Service [52] provided the first comprehensive review on glyphosate fate and effects related to forest uses in 1984, with a subsequent workshop proceedings pertaining to uses in coastal forests of western Canada constituting a second review [53]. Both documents provide detailed estimates of environmental exposures following normal use and concluded that such levels would be expected to have neither acute or chronic toxic effects, nor reproductive effects in animals. Durkin [37] published a more recent review and risk assessment in 2003, pertaining to typical ground-based backpack spraying of glyphosate at rates of 2.24 kg a.i./ha. The risk assessment generally supported the conclusions reached by the U.S. EPA, indicating that based on the currently available data, effects on birds, mammals, fish and invertebrates are minimal. Sullivan and Sullivan [54] provided another review of more than 60 published studies on glyphosate in forestry, considering potential effects of this management practice as a disturbance agent in forest ecosystems and focusing on aspects relating to biodiversity. These authors concluded that species richness and diversity of vascular plants, songbirds and small mammals were either not affected or affected to only a minimal degree by glyphosate treatments. The degree of change observed in all cases was considered to be within natural fluctuations. For both avian and small mammal species, temporary declines did occur in some species, whereas in other species, abundance actually increased in treated sites. Such differential responses are largely attributable to the specific habitat preferences of the species in question. For those species whose preferred habitat is removed by the herbicide treatment the typical response is transient reduction in populations in these specific treated sites, followed by return when these habitat features become re-established on the site. The impact of glyphosate on large mammalian herbivores was measured by abundance of animals and food plants and by habitat use. Hares (*Lepus* spp.) and deer (*Odocoileus* spp. and *Capreolus capreolus*) were little affected, whereas reductions in plant biomass and related moose (*Alces alces*) forage and habitat use generally occurred for 1 to 5 years after treatment. Studies on terrestrial invertebrates covered a wide range of taxa with variable responses in abundance to glyphosate treatments. The authors noted that management for a mosaic of habitats, which provides a range of conditions for plant and animal species, are likely to ameliorate any short-term changes in species composition which might occur on specific sites treated with glyphosate to enhance regeneration success and plantation growth rates following forest harvesting.

Several major field studies, as well as a hierarchical suite of lab to field studies focused on the effects of glyphosate on amphibian species, have been completed. Results of these studies provide a substantial empirical basis which taken as a whole demonstrates very low potential for significant direct deleterious effects of formulated glyphosate products on non-target organisms in forest ecosystems. One of the earliest of these studies was a long-term investigation conducted in the Carnation Creek watershed of coastal British Columbia. This whole ecosystem experiment involved a fall aerial application of glyphosate (Roundup) in which the herbicide was applied at a rate of 2.0 kg a.i./ha to 41.7 ha of the watershed. General results were summarized by Reynolds and co-workers [53, 55] with more specific details provided in a series of published studies by several of the principal investigators involved. A key focus of the study was on comparative fate and effects of the herbicide in directly over-sprayed versus buffered stream channels. Feng *et al.* [43] documented maximum glyphosate residues of 162 µg/L in stream water, 6.8 µg/g dry mass in bottom sediments and <0.03 µg/L in suspended sediments of two intentionally over sprayed tributaries, dissipating to <1 µg/L within 96 h post application. Buffered streams were characterized by very low glyphosate residue levels <4 µg/L in stream water. Ratios of maximum stream water concentrations of glyphosate observed in buffered and over sprayed tributaries relative to literature toxicity values indicated a substantial margin of safety under either operational or worst case scenarios. Holtby and Baillie [56] examined the responses of coho salmon (*Oncorhynchus kisutch*) fingerlings and observed some stress and low mortality of 2.6% in caged fish located in the over-sprayed tributary. No similar stress or mortality were observed in other sites. Catch per unit effort in the over-sprayed tributary declined immediately after the application but recovered within 3 weeks. While this was taken to suggest that coho fingerlings had been stressed by some component of the herbicide spray, no treatment related changes in over-winter mortality, growth rates, probabilities of entering and leaving the tributary or timing of spring emigration were observed in the two years subsequent to herbicide treatment relative to results from 1 to 3 years of pre-spray monitoring. Kreutzweiser [57] also concluded that herbicide treatments did not unduly disturb stream invertebrates. While drift densities of most aquatic invertebrates did not increase in response to herbicide applications, the slight increase in drift response of *Gammarus* sp. and *Paraleptophlebia* sp. observed downstream of treated areas may have resulted from herbicide treatment. Feng *et al.* [42] also documented fate and persistence of glyphosate and its primary metabolite in terrestrial compartments. Residues in red alder and salmonberry foliage were 261.0 and 447.6 µg/g respectively and indicated good impingement on the target. Leaf litter residues, which averaged 12.5 µg/g for red alder and 19.2 µg/g for salmonberry initially, declined to less than 1 µg/g within 45 days post application (DT50< 14 days). In soils, glyphosate and AMPA residues were retained primarily in the upper organic layers of the profile, with >90% of total glyphosate residue in the 0 to 15cm layer. Distribution data for both glyphosate and AMPA suggested strong adsorption and a low propensity for leaching. Glyphosate soil residues dissipated with time resulting in estimated DT50 values ranging from 45 to 60 days. After 360 days, total soil residues of glyphosate were 6 to 18% of initial levels. Results of the Carnation Creek study were consistent with a similar study conducted by Newton *et al.* [41] in the Oregon Coast range. Additional work in the latter study examined the exposure of mammalian herbivores, carnivores, and omnivores. Results showed that retention of the herbicide varied with food preference; however, all species had visceral and body contents at or below observed levels in ground cover and litter, indicating that glyphosate did not accumulate appreciably in animal tissues.

The Fallingsnow ecosystem project conducted in the boreal forest of northwest Ontario is one of the few studies to comparatively examine the ecological consequences of herbicide treatments, including glyphosate, with other methods of vegetation management. In this experiment, treatments included aerial applications of triclopyr ester (Release) at 1.9 kg a.i./ha or glyphosate (Vision) at 1.5 kg a.i./ha with direct comparison to mechanical cutting using either brush saws or tractor-mounted cutting heads. Lautenschlager [58] concluded that herbicide treatments had relatively inconsequential effects on most ecological response parameters examined in this boreal forest site. As part of this multidisciplinary study, Simpson *et al.* [59] observed no substantial treatment-related differences in the movement of selected nutrients such as total organic N, NH^{4+}, NO^{3-}, K, Ca. Woodcock *et al.* [60] assessed the effects on songbird densities as determined by territory mapping, mist netting, and banding and observed 20 to 38 species breeding within various treatment blocks. First year post-treatment assessments revealed that mean densities of the 11 most common species increased by 0.35/ha on the control plots. In contrast, densities on treated plots decreased by 1.1/ha (brush saw), 1.6/ha (Silvana Selective), 0.14/ha (Release) and 0.72/ha (Vision). A point of emphasis here is that essentially any effective vegetation management technique will alter available habitat to some degree. In at least this one study, songbird densities were relatively less impacted by herbicide treatments as compared to mechanical treatments. Response to these habitat changes will vary with species, favouring certain species while resulting in out-migration of other species at least for some period of time. As a single species example, chestnut-sided warbler (*Dendroica pensylvanica*) had lower (p <0.05) mean densities on the brush saw-treated and Silvana Selective-treated plots than on the control plots and fewer (p <0.05) female birds were captured in the first post-treatment year.

A particular strength of the Fallingsnow ecosystem project was the detailed studies on plant communities where relative differences were tracked before and 1 to 5 years after treatments. Newmaster and Bell [61] showed that species richness and abundance of pteridophytes, bryophytes and lichens were reduced by all of the silvicultural treatments. Herbicide applications had the greatest initial effect on species richness, species abundance, and diversity indices. The authors noted that cryptogam diversity showed signs of recovery 5 years after treatment and that missed strips or untreated areas within a clearcut, provided a refuge for remnant communities and could play a key role in the rehabilitation of sites in terms of recovering the full suite of plant diversity. Bell and Newmaster [62] further reported that woody, herbaceous and graminaceous species showed transient declines in species richness, abundance or foliar cover, diversity indices, and rank abundance, as would be expected given the intent of the treatment. As a result, spruce trees proliferated in the regenerating plantations, but in no case did single layer monocultures occur. While herbicides had a relatively greater initial effect on plant community composition as compared to the two different cutting treatments, woody, herb, and grass layers showed substantial resilience to all treatments and recovered to pre-treatment levels within five years. Duchesne *et al.* [63] examined effects on total captures, species richness, diversity, and assemblages of adult carabids (Coleoptera: Carabidae) and found no effect on total capture rates but an increase in species richness and diversity in response to all treatments.

As noted by Guynn *et al.* [12] impacts of forest-use herbicides on amphibians is an area that has been historically understudied. In recognition of this general dearth of scientific knowledge and the potential for both aquatic and terrestrial life stages of amphibians to be directly exposed to formulated glyphosate products, Thompson and co-workers undertook a multi-tier, hierarchical project including both laboratory and field component studies [64]. Each tier of study provided unique and valuable data pertaining to overall risk assessment for amphibians. The authors also noted the need to consider potential multiple stress and multiple species interactions in ecotoxicological research. As the lead component study in this series, Edginton *et al.* [65] reported 96-h LC10 and LC50 estimates ranging from 0.85 to 3.5 mg a.i./L for early larval stages (Gosner 25) of *Rana clamitans* and *R. pipiens*. These endpoints remain among the lowest documented toxicity endpoints for amphibians exposed to formulated glyphosate products. The study confirmed that amphibian larvae were more sensitive than embryos and showed general equi-sensitivity among the four amphibian species tested. Results also demonstrated that larval amphibians are among the most sensitive of aquatic organisms when exposed to formulated products of glyphosate containing the POEA surfactant and thus the importance of testing end-use formulations. Surfactants are generally required to be used with glyphosate to allow effective uptake of this electronically charged molecule across plant cuticles. Inclusion of the surfactant also results in reduced losses from treated foliage *via* rain-wash [43, 66]. The inclusion of the POEA surfactant in many formulations is also very important from an ecotoxicological perspective. It is well recognized that POEA is the primary toxicant to aquatic species. POEA and other surfactants may affect membrane transport generally and often act as a general narcotic [32, 33]. As such POEA mediated toxicity is well established as a concern for aquatic organisms such as fish and amphibians for which transport of oxygen and other compounds across gill or skin membranes is a critical physiological function. Unfortunately, owing to the chemical complexity of the POEA surfactant and resultant difficulty in analysing for it in complex environmental matrices, the environmental behaviour of POEA in natural forest ecosystems has not been specifically studied. However, fate experiments conducted in the laboratory show that the surfactant is also readily degraded in soils with a half-life of less than 7 days, that desorption from soil surfaces is minimal, and that persistence in natural waters under laboratory conditions resulted in an estimated half-life of about 2 weeks. Results of these studies suggested that POEA would be lost from the water column following application by a combination of sorption to sediments and microbial metabolism [67]. The half-life of POEA in shallow waters (15 cm deep) in the presence of sediments has subsequently been reported as about 13 h [68], further supporting the concept that any potential direct effects of formulated products on organisms in natural waters are likely to occur very shortly post-treatment rather than as a result of chronic or delayed toxicity.

Tier II studies conducted by Chen *et al.* [69] confirmed the interaction of pH and Vision toxicity in *R. pipiens* larvae and showed parallel effects for zooplankton population response parameters, suggesting that the pH–Vision interaction is of general ecological significance. In addition, Tier II studies demonstrated that effects on zooplankton reproduction could also be exacerbated by food deprivation when presented as a concomitant stressor. *In situ* enclosure studies conducted by Wojtaszek *et al.* [70] in two different wetlands systems showed 96-h LC10 and LC50 values generally higher than those derived from laboratory studies. This result was attributed to reduced magnitude and duration of exposures resulting from natural degradation and dissipation mechanisms which are active in real-world systems. Results clearly demonstrated the importance of including *in situ* manipulative studies in ecotoxicological risk assessments. Contrary to the results of the lab-based studies, the *in situ* enclosure experiment lead to the conclusion that typical silvicultural applications of Vision would not be likely to generate significant direct mortality in native amphibian larvae. This conclusion was strongly supported by both chemical and biological monitoring studies as reported by Thompson *et al.* [71] as the fourth and final tier of the research program. Results from these Tier IV studies showed no statistically significant differences in mean mortality among larvae of two different amphibian species (*R. clamitans* and *R. pipiens*) differentially exposed in over-sprayed, adjacent, and buffered wetlands. Results of the operational monitoring study were consistent with concentration-response relations from both Tier I and III studies since 99% confidence limits for real-world exposure concentrations in all wetland cases were below both estimated LC50 and LC10 values. As a general conclusion, results of this tiered research program indicate that aerial applications of the herbicide Vision, as typically conducted for conifer release in forestry, do not pose a significant risk of acute effects to the most sensitive aquatic life stages of native amphibians in forest wetland environments. The conclusion was consistent with specific risk assessments for formulated glyphosate products in aquatic systems [33]. Results of ongoing field studies consistently support this conclusion, thus allowing researchers to refocus their attention on more subtle but equally important potential effects on amphibian populations associated with possible indirect or multiple stressor interactions [72, 73].

Triclopyr

Triclopyr is the common name for ((3,5,6-trichloro-2-pyridinly)oxy)acetic acid, the active ingredient of formulated commercial products such as Garlon 3A and Garlon 4. These two products also represent two different chemical forms of triclopyr, that is the triethylamine salt and the butoxyethyl ester (BEE) respectively. Triclopyr mimics indole auxins as plant growth regulating hormones and causes plant mortality through induction of irregular cell growth, particularly in the stem tissues of vascular plants. Typical use rates for triclopyr are in the range of 4 kg a.i./ha, comparatively higher than those for glyphosate. Although triclopyr receives markedly less use in the international forest sector than glyphosate, it is a regionally important forestry herbicide in the southeastern USA and other areas where it is typically applied using ground-based techniques. The fate and effects of triclopyr in forest ecosystems have been previously reviewed [52]. In combination with data derived from several field studies conducted in a variety of forest ecosystems, it is well documented that triclopyr dissipates rapidly from foliage and soils. The primary degradation mechanism in soils is microbial and the principal metabolite is trichloropyridinol. Both laboratory and field study results suggest that triclopyr exhibits limited to moderate leaching or lateral mobility in soils [40, 74-76]. In aquatic compartments, BEE degrades *via* base-catalysed hydrolysis to yield triclopyr acid [77] which in turn may further degrade by either photolytic or biological means to yield the principal metabolite [52].

Wan [78], studied the comparative acute toxicity of Garlon 3A, Garlon 4, triclopyr, triclopyr ester, and their transformation products to juvenile Pacific salmonids, demonstrating that the ester was considerably more toxic than all other forms. The ester form of triclopyr is considered to be approximately 100 fold more toxic than the acid [79]. McCall [80], conducted simulations of the aquatic fate of triclopyr butoxyethyl ester emphasizing the importance of mechanisms converting the ester to less toxic forms as this is a critical determinant of potential toxic effects in fish such as coho salmon, as well as other aquatic organisms. Under low pH or cool temperature conditions, the transformation of ester to acid may be relatively slow and thus variations in these environmental parameters may strongly influence ecotoxicological outcomes. In this regard, toxicity of the ester form of triclopyr to fish, amphibians and aquatic invertebrates is the major concern in relation to potential ecological impacts and this aspect has received a substantial amount of scientific investigation.

Kreutzweiser and co-workers, conduced time-toxicity tests with rainbow trout (*Oncorhynchus mykiss*) under both laboratory and field studies. In flow-through toxicity tests [90] the effect of exposure time on the toxicity of triclopyr butoxyethyl ester (Garlon 4) to fish (rainbow trout, *Oncorhynchus mykiss*, and chinook salmon, *Oncorhynchus tshawytscha*) and stream insects (*Hydropsyche* sp. and *Isonychia* sp.). The toxicity of triclopyr ester to all species increased with increasing time of exposure to the ester. For example, median lethal concentrations for rainbow trout exposed for 1, 6, or 24 h were 22.5, 1.95, and 0.79 mg a.i./L of triclopyr ester. Results suggested that even under conditions where maximal predicted environmental concentrations (2.7 mg a.i./L) might occur, risk of acute toxicity would be very limited under typical exposure durations observed in flowing systems. In contrast, considerably higher risk of acute lethal effects could be predicted under conditions where the ester form might persist for more than 6 h, even when initial concentrations were as low as 0.7 mg a.i./L. The authors noted the aquatic organisms in lentic systems (such as wetlands, ponds and lakes) are likely to be most at risk. These relations were subsequently confirmed in various field studies.

A major multidisciplinary study focused on the ecotoxicology of triclopyr ester (Garlon 4) following aerial application at a rate of 3.84 kg a.i./ha that was conducted in a typical boreal forest watershed of northern Ontario, Canada. A particular focus of this study was on the fate and effects of the more toxic form of triclopyr (BEE) in the stream under a worst case scenario of direct overspray [81]. Results showed an average deposit at the stream surface of 3.67 kg a.i./ha with BEE residues in stream water exhibiting instantaneous maxima of <0.35 mg a.i./L. A series of diminishing pulses were observed resulting from direct inputs during overspray of the stream channel upstream. Average concentrations of the BEE in stream water ranged from 0.05 to 0.11 mg/L during the first 12 to 14 h monitoring period and were below limits of detection within 72 h. Both the average concentrations and exposure durations observed in this field study were substantially below levels generating acute lethal responses for various aquatic organisms in either lab or field studies [e.g. 83-87]. Initial whole body tissue residues in samples taken from fathead minnow cages *in situ* at the downstream location (43 mg a.i./kg) were similar to those predicted from simulation models [80]. No statistically significant mortality was observed in three species of aquatic organisms (yellow perch, caddisflies or fathead minnows) caged *in situ* either in treated or control areas. The authors concluded that natural dissipation mechanisms including photolysis, hydrolysis and microbial action limited exposures to sublethal levels and that based on this study, significant impacts to aquatic organisms would not be anticipated under operational conditions where such streams would be protected by buffer zones of 60 to 100 m. Similarly, in a field experiment in which trclopyr BEE (Garlon 4) was directly injected directly into a small headwater forest stream, intensive sampling [82] showed maximal aqueous concentrations of 0.848 and 0.949 mg a.i./L at the monitoring stations nearest two discrete injection points. Average BEE concentrations ranged from 0.32 mg a.i./L at stations nearest injection points to 0.02 mg a.i./L approximately 225 m downstream. Results demonstrated rapid conversion of the BEE to triclopyr acid in this system, as well as significant sorption of the chemical to natural allochthonous (deciduous leaf pack) materials. Resultant short-term, pulse-type exposures of BEE were observed with magnitude decreasing and duration slightly increasing with downstream distance. Resultant exposure regimes failed to induce any mortality of resident brook trout, nor were there significant effects on the growth of 1 or 2 year old brook trout.

In contrast to the results of lotic system experiments, substantial toxicity to a variety of aquatic organisms has been observed in lentic studies characterized by longer duration of exposure to the more toxic BEE form of triclopyr. Kreutzweiser *et al.* [83] conducted a dose-response study on fish caged within *in situ* enclosures in a northern Ontario lake. Results showed median dissipation times for aqueous residues ranging from 4 to 8 days. All caged rainbow trout exposed to initial concentrations greater than 0.69 mg a.i./L died within 3 days and 43% mortality was observed at 0.45 mg a.i./L whereas no mortality was observed at the 0.25 mg a.i./L level. Using similar *in situ* enclosures in two different wetland ecosystems, Wojtasek *et al.* [84] studied the effects of triclopyr BEE (Release) on mortality, avoidance response, and growth of larval amphibians (*Rana clamitans*, *Rana pipiens*). A range of treatment concentrations were applied to yield nominal concentrations ranging from 0.26 to 7.68 mg a.i./L. Concentration-dependent mortality and abnormal avoidance response were observed but there were no significant effects on growth. Toxicity for the two test species (*R. clamitans* and *R. pipiens*) were less than those observed in prior laboratory studies [85-87], probably due to the rapid dissipation of BEE which showed a DT50 of less than 1 day in both of these shallow wetlands. The authors noted that LC10 and EC10 endpoints approximated aqueous concentrations of 0.59 mg a.i./L that is within the range for expected environmental concentrations in small wetland amphibian breeding habitats under direct aerial overspray scenarios, thus presenting a potential risk of impacts for a small proportion of native amphibian larvae. This conclusion was consistent with results of laboratory microcosm studies in which Chen *et al.* [88] showed that triclopyr BEE (Release) at environmentally relevant test concentrations (0.25 and 0.50 mg a.i./L) resulted in significant decreases in survival of both larval life stages of *R. pipiens* and a common wetland zooplankton species *Simocephalus vetulus*. Moreover results indicated that effects on amphibians and zooplankton may be amplified by other concomitant stressors such as low food availability or low pH.

Overall, risk assessments for triclopyr BEE based on early tier experiments identified a substantial risk of acute toxicity to fish, amphibians, zooplankton and aquatic invertebrates, particularly in lentic systems where dissipation of the ester form is limited in some way. The presumption of risk was confirmed by subsequent field studies in scenarios where longer term exposure to the more toxic ester form occurred, but not in lotic scenarios where the duration of exposure to the ester was too short to attain toxic thresholds in aquatic organisms. Results emphasize the particular importance of understanding both the duration and magnitude of exposures that occur in real-world systems and the need for considering such natural exposure regimes when designing or interpreting research results and also when considering potential mitigative measures.

FOREST-USE INSECTICIDES

As compared to herbicides, fewer insecticides find widespread use in the forest sector internationally. Among those most commonly in use are the biological control agent *Bacillus thuringiensis* var. *kurastaki* (Btk) and the unique chitin-formation inhibiting chemical pesticide diflubenzuron (Dimlin®) (Table **1**). The use pattern for these products is highly sporadic with amounts applied varying dramatically in relation to the extent and severity of major insect pest outbreaks. Unlike herbicides, applications of insecticides are typically made to protect semi-mature or mature high value timber stands. Defoliating insect pests of significance historically in North America include the gypsy moth, spruce budworm, western spruce budworm, blackheaded budworm, jack pine budworm and Douglas fir tussock moth. Data provided by the USDA-Forest Service [16] indicates that of the total area treated for gypsy moth in the northeast region, 77% received applications involving Btk, while approximately 22% of the area was treated with Dimilin. In Canada, Btk is by far the most commonly used product accounting for approximately 86% of forest insecticide use [8], with the remainder being primarily tebufenozide (MIMIC). In the UK only four active ingredients were registered in 2004 as chemical insecticides for use in forestry [89]. Selected case studies for Btk and diflubenzuron are presented below to illustrate specific ecotoxicological issues of interest associated with forest uses of these active ingredients.

Increasingly, invasive insect species such as the mountain pine beetle, emerald ash borer, Asian long-horned beetle and brown spruce longhorn beetle are posing new and significant ecological and economic risks to the forest sector in North America [90, 91]. Similar invasive insect pest problems threaten forests in other countries, and often these are occurring in urban forest environments presenting several unique issues. For example, broadcast insecticide applications, as typically used against the major defoliating insect species, may be ineffective or publicly unacceptable as controls for invasive wood boring species. These issues have prompted the development and use of novel systemic injection techniques, as well as natural product insecticides such as azadirachtin [91] as alternative control techniques within broader integrated pest management strategies. A recent review [92] documents the environmental fate and effects information associated with several of these compounds which are purported to represent “reduced risk”.

Bacillus Thuringiensis Var. Kurstaki (Btk)

Among several strains of *Bacillus thuringiensis* with notable insecticidal activity, the proteinaceous crystalline toxin of Btk is known to be highly specific to larval Lepidoptera [93, 94]. The mechanism of action of Btk in Lepidoptera is the result of toxin induced rupture of the midgut followed by spore germination and septicemia in the body cavity that eventually results in death [95]. Several different formulations of Btk (see some examples in Table **1**), are used extensively in the USA and Canada, as well as for the control of Lepidopteran insect pests worldwide. One example of the latter is the use of Btk in an attempt to eradicate the invasive painted apple moth in New Zealand for which a comprehensive impact assessment has been published [96]. In North America, for major pests such as gypsy moth, spruce budworm, jack pine budworm and hemlock looper, applications are typically made by aircraft. Unlike conventional pesticides, the potency of Btk formulations is determined based on standardized bioassay response and reported in terms of Billions of International Units (BIUs). Typical application rates for Btk range from approximately 60 to 90 BIU/ha.

Results of published risk assessments [18, 96] indicate that given their highly specific mode of action, Bt products are unlikely to pose a significant hazard to vertebrates, fish, birds or insects other than macrolepidopteran larvae. Bt occurs naturally in soils throughout the world. The vegetative form of Btk does not generally persist in soil; however, endospores can survive in most types of soils for extended periods with half-lives of spores usually in the range of 100 to 200 days [97]. As noted in the New Zealand environmental impact assessment document [96], estimates on persistence of Bt toxins vary widely and there is some evidence to suggest that binding of Bt toxins to humic acids, organic supplements or onto soil particles protects the toxins from microbial degradation, without eliminating their insecticidal activity. Leaf litter and soil samples collected following aerial spray Foray 48B for control of white-spotted tussock moth in Auckland, showed significantly enhanced levels of Btk-like isolates up to two years post-spray. Laboratory studies by Visser and other workers [98], had previously shown that formulated Btk products generally had no effect on functional parameters associated with soil microflora.

The New Zealand environmental impact assessment generally suggested that relative to all available options, Btk was likely to be the most acceptable approach for attempted eradication of painted apple moth in the urban area of Auckland, from a public, economic, efficacy and environmental perspective. The World Health Organization specifically concluded that "Bt products may be safely used for the control of insect pests of agricultural and horticultural crops as well as forests". While such comprehensive assessments typically support the use of Btk as environmentally acceptable, there are concerns associated with potential ecotoxicological impacts on non-target Lepidoptera and derivative indirect effects on insectivorous species, particularly birds, which may depend upon these organisms as a primary food source, as well as potential effects on non-target aquatic insects.

Several studies demonstrate that Btk causes immediate reductions in abundance and species richness of non-target larval Lepidoptera [99-102]. Butler and co-workers [103] conducted extensive studies on this aspect following applications of Btk for control of gypsy moth in oak forests of West Virginia, USA. During the treatment year, Btk produced significant decline of canopy-dwelling macrolepidopterous larvae. No differences in abundance of various caterpillar species were observed among treated and control plots during the weeks or months following treatment. Similarly, no difference between treated and control plots were observed in abundance of most species in 1992, the first post-treatment year. Non-lepidoptera species also appeared to be unaffected by the Btk treatment. The fact that abundance and richness of non-target lepidopteran larvae declined during each year of the three year study, even on non-treated plots, emphasizes the importance of using appropriate controls in field studies of this type. It also underscores the need to consider pesticide-induced perturbations in light of the natural variation in abundance that may occur due to both random and non-random factors which typically influence biological systems in natural environments.

Boulton *et al.* [99] assessed the impacts of Btk (50 BIU/ha as Foray 48B) on native, non-target Lepidoptera following treatments to 12,805 ha of Garry oak forests for control of gypsy moth in southeastern Vancouver Island, British Columbia, Canada. Significant variation in diversity among the Lepidoptera were not detected, but reduced richness and abundance on two different host plant species were observed. The authors noted potential concerns associated with such effects, particularly in highly fragmented forest stands such as those associated with urban or industrial areas. They also emphasized the importance of such effects on rare and endangered non-target lepidopteran species such as *Euchloe ausonides isulanus*, and *Euphydryas editha taylori* which are found only in oak meadows and rocky knolls. In a follow-up study, Boulton *et al.* [100] examined longer term recovery of non-target Lepidoptera noting that reductions were greatest one year post-treatment. Relative to the reference sites, each of 11 species that were initially reduced by the Btk applications showed an increase in the treatment sites within the next 3 years, by which time only four species remained significantly reduced in the treatment sites. The uncommon species were significantly reduced in the year of treatment but not one or three years post-treatment. Results of this study highlight the importance of long term monitoring following pesticide induced perturbations in relation to understanding the rate and process by which recovery in ecosystem structural or functional processes may occur. The study also emphasizes the important consideration of effects on rare and endangered species. In general, and somewhat surprisingly, this aspect has does not appear to have received sufficient scientific attention. In the case of Btk, such a concern has been raised for the Karner blue butterfly. Herms *et al.* [104] conducted a field survey and laboratory bioassay demonstrating significant dose-dependent mortality in response to Btk treatments and found that early and late instars were equally susceptible. The authors concluded that the Karner blue is both phenologically and physiologically susceptible to Btk as employed for gypsy moth suppression, even though the larval generation at risk and extent of phenological overlap may vary from year to year.

In cases where direct effects on species in one trophic level deleteriously affect those in other trophic levels, concerns over ecological implications are heightened. In the case of forest-use insecticides with highly specific modes of action, such as Btk, potential indirect effects of reduced prey or food availability on insectivorous predators are of particular interest. Two separate studies [105, 106] have examined such indirect effects on insectivorous birds and small mammals in Ontario, Canada. As would be anticipated, substantial reductions in

Lepidoptera larvae were observed in response to treatment. Many adult male shrews apparently emigrated and were replaced by young males and females. Effects on Nashville warbler and hermit thrush, chicks of spruce grouse, and adult male masked shrew were all attributed to indirect effects associated with reduction in the primary insect food source. Holmes *et al.* [105] further examined the hypothesis that food reductions caused by forest spraying with Lepidoptera-specific insecticides would affect songbird behaviour and reproduction. The comparative study of Tennessee warbler nests and parental behaviour involved spray blocks treated with Btk, tebufenozide (MIMIC) or left as an untreated control area. Nestling survival and growth were unaffected by the insecticide treatments. Nests in the treated blocks had smaller clutches, smaller broods and lower hatch rates than nests in the control block, but these differences were not statistically significant. Nestling diets were similar in the MIMIC and control blocks. There were slight differences in the behaviour patterns of female Tennessee warblers in the MIMIC and control blocks, with those from MIMIC treated spending less time at the nest and more time foraging. The authors concluded that the indirect effects of forest spraying with Lepidoptera-specific insecticides pose little risk to forest songbirds. Differential results may reflect species-specific behaviours, food preferences and ability to prey switch or broaden foraging ranges. The equivocal nature of these field study results, suggests that strategic species-specific biomonitoring and population modeling in conjunction with operational spray programs may be warranted to provide more conclusive evidence with regard to possible ecological consequences at broader spatial scales. Other studies have also documented potential indirect effects of Btk spraying associated with reductions in natural food for breeding black-throated blue warblers [107] and an endangered species – the Virginia big-eared bat [108].

Kreutzweiser *et al.* [109, 110] conducted a series of studies to examine the effects of Btk, as two different aqueous formulations of Dipel, on several aquatic invertebrate species (various species of Ephemeroptera, Plecoptera or Trichoptera). Results showed no significant mortality, drift response or consumption of treated leaf disks at levels well above label rates or expected environmental concentrations using either flow-through laboratory experiments or outdoor stream channel experiments. Although trends of reduced decomposition activity in treated outdoor stream channels were observed, there were no significant differences in mass loss of leaf material between treated and control channels. These results from laboratory and controlled field experiments indicated that contamination of watercourses with Btk is unlikely to result in significant adverse effects on aquatic invertebrates or microbial community function in terms of detrital decomposition. In a confirmatory field study, Kreutzweiser *et al.* [111] treated a section of a natural forest stream with Btk at 10 times the expected environmental concentration (200 BIU/mL) to determine effects on the aquatic macroinvertebrate community. Invertebrate drift density increased slightly, but only during the 0.5-h application and only at the site 10 m below the application point. There were no significant changes in taxonomic richness of benthic invertebrates after the application, but there were short-term alterations in community structure at the treated site after the application, as measured by a dissimilarity index. In 11 of 12 benthic taxa for which there were sufficient data, changes in abundance after the application were not significant compared with changes in abundance at the reference site. The stonefly *Leuctra tenuis* (Pictet) was reduced by ~70% at the treated site 4 days after the application, and abundance of this stonefly remained considerably lower, but not significantly different, from the reference site for at least 18 days. A follow-up study demonstrated that under laboratory conditions, Btk on leaf material was not toxic to *L. tenuis.* The Btk application had no significant effect on the growth or survival of caged caddisfly larvae, *Pycnopsyche guttifer*, in the treated stream.

Diflubenzuron

Diflubenzuron is a benzoyl-phenylurea insecticide that inhibits chitin deposition in arthropods. It is effective either as a stomach or contact insecticide. In forestry, diflubenzuron sees major use principally in the USA for suppression of gypsy moth. Two formulations (Dimilin 4L and Dimilin 25W) are registered in the USA and the active ingredient is also efficacious against tent caterpillar and several other forest insect species including pine false webworm [112] and eastern hemlock looper [113]. For suppression of gypsy moth, diflubenzuron may be applied *via* either ground or aerial methods at rates ranging from 9 to 75 g a.i./ha. Typical use scenarios under severe infestation conditions may involve multiple applications over several years.

In a synoptic review of potential environmental effects of diflubenzuron [114], adverse effects on crustacean growth, survival, reproduction, and behaviour have been observed at environmentally realistic levels ranging from 0.062 to 2 μg/L. Rebach and French [115] examined the effects of diflubenzuron on blue crabs and provide a review of potential effects in marine and estuarine environments. This review demonstrated substantial toxicity to these species. Surprisingly, there appear to have been no field studies investigating potential effects on freshwater crayfish which are likely to be directly exposed during forest use. Mayflies, chironomids, caddisflies, and midges also have known sensitivity to diflubenzuron at similar aqueous concentrations, showing low emergence and survival as typical impacts. Fischer and Hall [116] reviewed the environmental fate and effects data on diflubenzuron with particular emphasis on aquatic systems. Organic matter and aquatic macrophytes are major factors influencing the adsorption and degradation of the compound. Reardon [117] presented an overview of field experiments examining the potential impacts of diflubenzuron (Dimilin 4L) on selected non-target organisms in an experimental broadleaf forest in West Virginia. Five non-target groups were monitored, including: fungi, bacteria, and invertebrates in leaf litter and soil; aquatic macroinvertebrates; canopy arthropods; pollinating insects and aquatic and terrestrial salamanders. Initial concentrations of diflubenzuron and degradation of residues on tree surfaces, in leaf litter, in soil, and in water were also determined. Except for aquatic macroinvertebrates, canopy arthropods, and native pollinating insects, there were no detectable effects of the treatment on the non-target groups. Diflubenzuron treatments were shown to decrease the densities and survival of several species of mayflies, stoneflies and a cranefly and reduce richness and abundance of non-target terrestrial arthropods, primarily macrolepidopterans and yellow jackets. Durkin [118] characterized the scientific database supporting the risk assessment of diflubenzuron (Dimlin) in forestry as large and somewhat complex, but concluded that direct effects of diflubenzuron on mammals, birds, amphibians, fish, terrestrial and aquatic plants, microorganisms, and non-arthropod invertebrates were considered implausible, largely owing to the specific mode of action for this compound. Just as for Btk, the fact that diflubenzuron is an effective insecticide against Lepidoptera results in a substantial likelihood of effects on other non-target members of this group, as well as indirectly on insectivorous species such as birds, which may specifically rely on these insect populations as their primary food source. Potential effects on aquatic invertebrates were also considered possible depending upon site-specific conditions controlling deposition to surface waters and thus resultant exposure levels.

Numerous laboratory studies have demonstrated the sensitivity of aquatic invertebrate species to diflubenzuron. Hansen and Garton [119] showed that among complex stream faunal communities in the laboratory, mayflies and stoneflies were affected at 1.0 µg/L and that crustaceans were also particularly sensitive. These authors also noted that single species toxicity tests adequately predicted direct lethal effects, but not indirect effects resulting from altered interspecies interactions. Liber *et al.* [120] conducted an elegant field mesocosm experiment using a concentration-response design approach and derived field EC50 values for insect emergence inhibition ranging from 1.0 to 1.4 µg/L. Overall, they concluded that significant adverse effects on insect emergence could be expected at diflubenzuron concentrations of >1.0 µg/L with the time to recovery being concentration dependent. Boyle *et al.* [121] also employed outdoor mesocosms in a study exploiting the unique mode of action of diflubenzuron to examine the indirect responses following direct impacts at the primary consumer (*i.e.* invertebrate) trophic level. Direct reductions in invertebrate grazers caused indirect increases in algal biomass. Indirect effects including 50% reductions in biomass and in individual weight of juvenile bluegill occurred because of apparent decreases in invertebrate food resources. In contrast, no statistically significant impacts were observed on adult bluegill or largemouth bass for the duration of the experiment. Results indicated that diflubenzuron had both direct and indirect impacts on the experimental aquatic ecosystems under the conditions tested and although treatment regimes were not environmentally realistic in relation to forest use patterns, the study provides an excellent example of potential indirect secondary and tertiary effects in aquatic systems that would be difficult if not impossible to determine under laboratory conditions. In a semi-operational field study in mixed wood forests of central Ontario, Canada, Sundaram *et al.* [23] reported that the fate of diflubenzuron residues following aerial applications of Dimlin 25W at a rate of 0.07 kg a.i./ha. Results indicated that dissipation patterns differed among water, sediment and aquatic vegetation substrates, with reported DT50 values of <1.3 days in pond water and <14 days in all cases. Zooplankton and benthic invertebrate populations were monitored for up to 110-day post-spray in two over-sprayed ponds with comparison to control ponds. Significant mortality occurred in two groups of caged macroinvertebrates (amphipoda and immature corixidae) 1 to 6 days post-treatment. Three taxa of littoral insects (*Caenis*, *Celithemis* and *Coenagrion*) were also significantly reduced in abundance in the treated ponds 21 to 34 days post-treatment, but recovered to pre-treatment levels by the end of the season. Zooplankton (cladocerans and copepods) populations were reduced 3 days after treatment and remained suppressed for 2 to 3 months.

Harrahy *et al.* [122] noted that diflubenzuron may persist on hardwood leaves throughout the growing season up until the time of leaf fall. Non-target aquatic organisms that consume these fallen leaves may therefore be exposed to the pesticide for a significant period of time. Several field studies have further investigated the potential effects of diflubenzuron on sensitive non-target aquatic invertebrates and confirmed effects under environmentally realistic scenarios. Griffith *et al.* [123] used Malaise traps to monitor emergence and flight distances of adult Plecoptera and Trichoptera from headwater streams in two different catchments of an experimental forest in West Virginia, USA before and after application of diflubenzuron. Stonefly, *Peltoperla arcuata* emergence was reduced in the first 4 months after treatment, as compared with the untreated catchments, however no differences in emergence of other species were observed. In a follow-up study [124], the flight of the stonefly *Leuctra ferruginea* was reduced in the treatment watersheds compared with the reference watersheds during the year following abscission of the treated leaves. Adult flight of other species did not decrease in the treatment watersheds during 1993. These results suggest that among aquatic invertebrates, stoneflies may be particularly sensitive to the effects of diflubenzuron even under scenarios of a single application. The authors noted that multiple applications of diflubenzuron over several years, which often occurs during gypsy moth suppression programs, may present a significant risk to these aquatic species. Similarly, Hurd *et al.* [125] observed significant reductions in the abundance of several taxa in treatment as compared to control watersheds following aerial application of diflubenzuron (Dimlin 4L) at a rate of 70 g a.i./ha. Most affected taxa included the stoneflies, *Leuctra* sp. and *Isoperla* sp., the mayfly, *Paraleptophlebia* sp., and the cranefly, *Hexatoma* sp. In a functional context, shredders were the dominant group affected, with reduced mean densities in treatment watersheds whereas densities of species such as Oligochaeta and Turbellaria increased in streams in treated watersheds. The authors again emphasized that since most aquatic insects oviposit in the watershed from which they emerge, repeated applications of diflubenzuron could have longer-term localized effects on invertebrate fauna in treated streams. In contrast to these studies, Boscor and Moore [126] studied the impacts of Dimilin at 70 g a.i./ha to a one-half mile stretch of White Deer Creek in central Pennsylvania. No spray-induced, adverse effects were detected on the organisms sampled, principally Ephemeroptera, Chironomidae, Trichoptera, and Plecoptera, for a period of up to 28 days after treatment.

The potential effects of diflubenzuron on non-target terrestrial insects have also been extensively examined. Sample [127] reported that the operational application of Dimilin [70, 75 g a.i/ha] resulted in greatest impacts on Lepidoptera which displayed reduced abundance and species richness at treated sites. No effects were observed among Coleoptera, Diptera, or Hymenoptera. Butler *et al.* [128] summarized results of a 6-year study conducted to evaluate the impact of diflubenzuron on the diversity and abundance of arthropods in West Virginia. Based on foliar sampling, overall arthropod family diversity and abundance, numbers of macrolepidoptera and beetles were significantly reduced in treated watersheds. Total arthropod abundance and macrolepidoptera abundance remained at significantly lower levels up to 27 months post-treatment. As noted by Durkin [126] some secondary effects resulting from reduced Lepidoptera prey may include increased foraging range, relocation and lower body fat content among foraging birds species. For example, Whitmore *et al.* [129] showed significantly lower fat reserves in seven of nine tested bird species following Dimlin applications to forests in the USA. Possible causal factors were listed as reduction in food availability and decreased biomass ingestion, increased energetic expenditures required in obtaining scarce food and reduced food quality in treated as compared to control sites. The latter study is an example of an investigation on functional (community energetics) rather than structural effects of pesticide use in forest ecosystems, an area which is generally under-studied. Another example is the study by Paulus *et al.* [130], who compared three methods for assessing the impacts of forest-use insecticides diflubenzuron and Btk on biological activity of soil organisms. While results were dependent on the monitoring technique employed, overall findings demonstrated transient effects on biological activity of soil organisms exposed to diflubenzuron but not Btk.

CONCLUSIONS

The cumulative wealth of scientific data available for modern forest-use herbicides and insecticides is extensive. Research spans multiple tiers of testing ranging from simple laboratory studies, through microcosm and *in situ* mesocosm studies and includes several comprehensive large scale field experiments. Higher tier field studies provide several unique benefits that are considered highly contributory to comprehensive ecotoxicological risk assessments. As many previous authors have suggested, it is impossible to replicate natural ecosystems, inclusive of all of their innate and interactive physical, chemical and biological components in the laboratory. In ecotoxicological risk estimation, direct use of data from any laboratory study carries the critical and highly questionable assumption of equivalence of the test system and the real world. As such, it is very prudent to continue the use of *in situ* mesocosm, manipulative field studies and long-term monitoring to confirm that extrapolative predictions based on early tier laboratory studies are in fact valid. In higher tier field studies, experimentation should be focused on typical operational as well as worst case maximal use rates with common end-use products such that results incorporate any potential effects associated with surfactants or other formulants contained therein. In terms of response variables, these should be focused on population or community level response and recovery time, as these are typically most relevant to regulatory and policy decision making and involve levels of biological organization and interaction mechanisms (e.g. predation, competition, commensalism) that cannot be effectively simulated in laboratory experiments. Examination of the case studies presented here highlight all of these unique benefits as well as the overriding value of large scale field experiments in terms of negating or confirming risk. While these benefits and values are particularly important in forestry scenarios owing to the typically large scale of operations, similar advantages apply to field experimentation in other sectors as well.

Given the relatively specific mode of action of many modern pesticides, their general high water solubility and facile environmental degradation and metabolism, environmental concerns associated with modern forest-use pesticides differ significantly from historic issues associated with mass mortality, long-term persistence and bioaccumulation. Potential ecotoxicological impacts associated with modern day synthetic, natural product and biological pest control agents are likely to be much more subtle and are commonly associated with indirect or secondary effects associated with habitat alteration, reduced food resources or multiple stress interactions. For glyphosate and Btk, respectively the dominant herbicide and insecticide used in the forest sector internationally, case study evaluations reviewed here support the conclusions of several more comprehensive risk assessments. Based on the weight of scientific evidence currently available, these data and risk assessments suggest that the judicious use of these products, in accordance with product labels, pose little risk to forest environments or non-target wildlife species. In contrast, higher tier field studies conducted with triclopyr ester and diflubenzuron, confirm specific risks under environmentally realistic or operational conditions imposing a requirement for mitigative actions sufficient to negate the risk. In such cases it is considered prudent to use adaptive management strategies, including operational chemical and biological monitoring of small scale operational test programs to ensure that mitigative actions do in fact protect sensitive values and general ecological integrity of receiving environments. A highly positive sidelight of detailed operational monitoring studies is the ability to generate both exposure and effects data critical to effective probabilistic risk analysis. Overall, case study analyses presented here support the continued judicious use of pesticides as part of sustainable forest management. In cases where risks are identified, appropriate mitigative measures may still allow them to be employed where no other effective options exist.

With emphasis that the scientific knowledge base associated with potential ecotoxicological effects of major forest-use pesticides is both extensive and detailed, there are, as always, some areas where further research would be considered particularly valuable. These focus areas include: (a) evaluation of potential interactive effects of tank-mixed herbicides; (b) assessment of plausible multiple stressor interactions (e.g. chemical and concomitant drought stress); (c) investigations on impacts on key ecosystem functional processes; (d) development and application of cost-effective operational monitoring techniques applicable over broader spatial scales and longer time frames than typical empirical studies; (e) application of probabilistic analyses and; (f) risk assessments that include population modelling over larger spatial and temporal scales.

Relative to the available information base on forest-use pesticides, our scientific knowledge on potential impacts of alternative vegetation or insect pest control techniques is exceedingly weak. As noted by Scriber [131], all pest management programs carry some risk of negative environmental impacts; this includes the “do nothing” option. In general, it is inappropriate to assume that biological controls, natural pesticides or other non-chemical approaches pose no risks to ecological integrity of forest ecosystems. In fact there are several lines of evidence that clearly demonstrate this assumption to be invalid – see Thompson and Kreutzweiser [92] as but one example. It is imperative that all options with potential use in integrated pest management strategies be equally scrutinized against cost, effectiveness and environmental acceptability criteria. One, particularly valuable means of conducting such comparisons is through direct side-by-side multi-disciplinary field studies conducted at semi-operational or operational scales. Finally, from the ecological perspective alone, the potential deleterious effects associated with the “do nothing option” or with the use of ineffective options may in fact be greater than those associated with pesticide treatment or other pest control alternatives. As a generality there appears to be far too little scientific or policy attention paid to this aspect. Similarly, there may also be significant economic implications of weakly effective or non-intervention strategies. Multiple cases of exotic invasive plant or insect pests as currently extant in the North American forest sector and elsewhere around the globe are unfortunately providing demonstrable evidence supporting the point that ineffective or non-intervention options are often not acceptable in either ecological or economic terms. Within an integrated management strategy, those options which best meet the three fundamental criteria of efficacy, economics and environmental acceptability should be made available and used by resource managers in optimizing the twin goals of sustainable resource use and protection of ecological integrity. All organisms, including humans, as integral components of forests and other global ecosystems are ultimately dependent on the successful achievement of those goals.

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