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Coca and Poppy Eradication in Colombia: Environmental and Human Health Assessment of Aerially Applied Glyphosate

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Contents

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I. Introduction

It is estimated that some 200 million people worldwide use illicit drugs. Most of these drugs have natural origins, such as cannabis, cocaine, and the opiates; however, the synthetic drugs such as the amphetamines also comprise a significant proportion of these uses (UNODC 2003). In response to the socioeconomic impacts of the production and distribution of illicit drugs, a number of individual nations, as well as multinational organizations, have initiated programs to reduce and eventually eliminate their production and distribution (UNODC 2003).

Coca (*Erythroxylum coca* and related species) is commonly associated with the tropical regions of South America. A number of species of coca are found in South America, and various varieties grow in the wild or are cultivated in different climatic conditions. It is primarily found in regions with temperatures above 25°C and with rainfall >1000 mm/yr. Currently, it is widely cultivated in Colombia, Bolivia, and Peru, with some cultivation in Ecuador, Venezuela, Brazil, and Argentina. In 2002, it was estimated that, of the 800 t cocaine produced in Latin America, 580 t was produced in Colombia, a reduction of about 100 t from 1999 (UNODC 2003). It is estimated that, worldwide, about 14 million people abuse cocaine (UNODC 2003).

Opium, morphine, and its derivative, heroin, are produced from the poppy *Papaver somniferum,* which is primarily grown in Asia. Global production of opium in 2002 was estimated to be 4,500 t, of which about 97 t was produced in South America; of this, 50 t was produced in Colombia (UNODC 2003). It is estimated that, globally, about 15 million people use opiates and that about 10 million of these use heroin (UNODC 2003). Similar to coca, the use of opium and morphine has historical roots in the traditional society of the producer regions but became more widely used as a human medicine when introduced to other parts of the world. Although morphine is still used for medicinal purposes, heroin use is largely illegal and its production and distribution have significant socioeconomic impacts in producer and consumer nations.

The growing and production of illicit drugs in Colombia have significant political, social, economic, and environmental impacts. While recognizing the importance of the political, social, and economic aspects of the issue, this review is focused on the human health and environmental significance of growing these crops and the control of coca and poppy through the use of the aerially applied herbicide glyphosate.

It is important to recognize that the actual production of coca and poppy as well as the processing and production of cocaine and heroin involve significant environmental impacts. Both coca and poppy are grown intensively in a process that involves the clearing of land, the planting of the crop, and its protection against pests including weeds, insects, and pathogens. Depending on the region, the clearing of the land for production purposes may have large and only slowly reversible effects on the environment. As for other forms of agricultural production, the clear-cutting of forests for the purposes of coca and poppy production reduces biodiversity, contributes to the release of greenhouse gases, increases the loss of soil nutrients, and promotes erosion of soils. Because production is illegal, it usually takes place in remote locations. As a result, clearing of land is done without government approval and with little apparent consideration for the biological and aesthetic value of the ecosystem.

A number of pesticides are used in the production of illicit drugs. Herbicides may be used in the initial clearing of the land and later in the suppression of weeds. Similarly, insecticides and fungicides may be used to protect the illicit crops from pests and diseases. To increase yields, fertilizers and other nutrients may also be used. Large quantities of agrochemicals have been seized and confiscated as part of the program to control the production of illicit drugs (Dirección Nacional de Estupefacientes 2002). Although some of these agrochemicals are highly toxic to mammals and may have significant environmental impacts, accurate information on the type of formulation used, amounts used, their frequency of use, and the conditions of their use is not available. Because of this, it was not possible to conduct a detailed human health and ecological risk assessment. However, the relevant toxicological and environmental properties of these substances are summarized in two separate reports, and several of these are significant potential hazards to human health and the environment (CICAD/OAS 2004c, 2005).

In addition to the use of agrochemicals in the production of coca and poppy, large amounts of chemicals are used in the processing of the raw product into refined cocaine and heroin. Processing of the illicit drugs is conducted in remote locations and in the absence of occupational health and environmental regulations and controls. During and after use, these substances may be released into the environment and have significant impacts on human and animal health and the ecosystem. The toxicological and environmental properties of these substances are summarized in a separate Tier 1 Hazard Assessment Report (CICAD/OAS 2004c). Some of these substances have potentially large environmental and human health hazards, and a subset of these is discussed in more detail in a Tier 2 Hazard Assessment Report (CICAD/OAS 2005).

The growing of coca and poppy and the distribution of cocaine and opium/heroin in Columbia have been the focus of a national control and eradication program starting in the 1970s. The program involves a number of departments and agencies of the Colombian Government and is coordinated by the Dirección Nacional de Estupefacientes (DNE), an agency of the Ministry of the Interior and Justice. The program has three main foci: the control of production of coca and poppy, the control of the processing, purification, and transport of the cocaine and heroin, and the seizure and forfeiture of the profits of illicit drug production (Dirección Nacional de Estupefacientes 2002).

The eradication program for illicit crops in Colombia is the responsibility of the Antinarcotics Directorate of the Colombian National Police (DIRAN-CNP), supported by data gathering from other nations such as those in North America and Europe. The DIRAN conducts regular flights with aircraft that spray coca and opium poppy crops with a herbicide. The DIRAN reviews satellite imagery and flies over growing regions on a regular basis to search for new coca and opium poppy growth and to generate estimates of the illicit crops through high-resolution low-altitude imagery and visual observation. The DIRAN selects the locations of the illicit crops that are to be sprayed with input from the DNE or the Government of Colombia's Plan Colombia Office.

Several concerns have been raised about the use of glyphosate and adjuvants in the control of coca and poppy plants. These concerns range from damage to other crops to adverse effects on the environment and human health. In response to this, the Government of Colombia appointed an independent environmental auditor who reviews the spray and no-spray areas with the DIRAN and regularly monitors the results of spraying through field checks and analysis of data from computerized spray records.

The objectives of the present assessment and review are to provide a science- and data-based study of the use of glyphosate in the eradication program with a key focus on the environment and human health, to collect data for use in the assessment, and to address specific concerns that have been raised. As with all risk assessments, we have followed a framework based on those used in other jurisdictions (NRC 1986; USEPA 1992, 1998). This framework consists of a Problem Formulation, Effects and Exposure Assessment, and Risk Characterization for both humans and the environment. In conducting this review, we used data from the peer-reviewed scientific literature, from government documents, and from studies specifically conducted to address data gaps.

II. Problem Formulation

Problem formulation is a key step in the risk assessment process and places the use of the substances being assessed into a local context. It is recognized that the growing of illicit crops such as coca and poppy, as well as the refining of the cocaine and heroin, involves considerable impacts on the environment through clearing of forests and the use of a number of substances for promoting crop growth and refining of the drugs. Although the identity of these substances is known, the type of formulation, the quantities used, and their manner of use is largely unknown and exposures in workers cannot be easily estimated. While the hazard of these substances is known (CICAD/OAS 2004c, 2005), the risks cannot be estimated, as the logistics of collecting the human and environmental exposure data are very difficult and not without other risks. Because of this, and as it was the initial mandate of the Panel, the focus of this risk assessment is on the use of glyphosate and adjuvants for control of the illicit crops. In this case, the locations and amounts of application are known with accuracy and environmental risk can be better estimated.

In humans, there are no specific biomarkers for exposure to glyphosate that can be used to estimate historical exposures. For logistical reasons, it was not possible to measure exposures resulting from eradication spraying directly in the field. For that reason, in epidemiology studies, indirect measures of exposures such as ecological studies, where the indicator variable or exposure is defined by eradication spraying and crops production patterns, must be used.

A. Stressor Characterization

The potential stressors in this risk assessment are glyphosate, its formulants, and adjuvants, such as surfactants, that are added to the spray formulation to modify its efficacy. The properties of glyphosate and these substances are described in the following sections.

Glyphosate

Glyphosate is the active ingredient of a number of herbicide formulations and is one of the most widely used pesticides on a global basis. Uses include agricultural, industrial, ornamental garden, and residential weed management. In agriculture, the use of glyphosate is increasing and use in soybeans is significantly greater since the introduction of glyphosate-tolerant crops (Wolfenbarger and Phifer 2000). According to the U.S. National Pesticide Use Database (USNPD 2006), use in soybean increased by 330% between 1992 and 1997 and by 460% between 1997 and 2002. Other agricultural uses for glyphosate-based products include its use by farmers as a routine step in preplanting field preparation. Nonagricultural users include public utilities, municipalities, and regional transportation departments where glyphosate is used for the control of weeds or noxious plants. The environmental and human health properties of glyphosate have been extensively reviewed (Giesy et al. 2000; Solomon and Thompson 2003; Williams et al. 2000) and by regulatory agencies (NRA 1996; USEPA 1993a, 1997, 1999; World Health

Organization International Program on Chemical Safety 1994). The following sections highlight key issues with regard to those properties of glyphosate that are fundamental to the assessment of risks associated with the coca and poppy eradication programs in Colombia.

Structure and Chemical Properties. The most common technical form of glyphosate is the isopropylamine salt (IPA), *N*-(phosphonomethyl) glycine isopropylamine salt (MW, 226.16; CAS number, 1071-83-6). The chemistry of glyphosate is important in determining its fate in the environment. Glyphosate (Fig. 1) is a weak organic acid comprising a glycine moiety and a phosphonomethyl moiety and closely resembles naturally occurring substances. Glyphosate is not chemically reactive, is not mobile in air or soils, does not have great biological persistence, and does not bioaccumulate or biomagnify through the food chain (CWQG 1999; Giesy et al. 2000; USEPA 1993a; Williams et al. 2000; World Health Organization International Program on Chemical Safety 1994).

Glyphosate is readily ionized and, as the anion, will be strongly adsorbed to organic matter in soils of normal pH. It thus has little mobility in soils and is rapidly removed from water by adsorption to sediments and suspended particulate matter (Giesy et al. 2000).

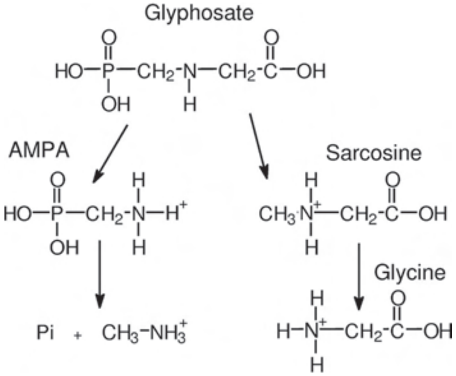


Fig. 1. Structure of glyphosate and its major metabolic and breakdown products.

Mechanism of Action. The mechanism of action of glyphosate is via the inhibition of the enzyme 5-enolpyruvyl shikimate-3-P synthetase, an essential enzyme on the pathway to the synthesis of the aromatic amino acids in plants (Devine et al. 1993; Franz et al. 1997). This inhibition results in decreases in the synthesis of the aromatic amino acids tryptophan, phenylalanine, and tyrosine, as well as decreased rates of synthesis of protein, indole acetic acid (a plant hormone), and chlorophyll. The death of the plant is slow and is first seen as a cessation of growth, followed by chlorosis and then necrosis of plant tissues. Inhibition of 5-enolpyruvyl shikimate-3-P synthetase is specific to plants. Many animals obtain their aromatic amino acids from plants and other sources and do not possess this pathway of synthesis. For this reason, glyphosate is relatively nontoxic to animals but is an effective herbicide in plants.

Global and Local Registration and Use. Glyphosate has been registered since 1971 and is currently widely used as a broad-spectrum, nonselective, postemergence herbicide in a number of countries around the world (World Health Organization International Program on Chemical Safety 1994). It is rapidly translocated from the leaves of treated plants to other parts of the plant, including the growing tips of stems and roots, and to underground storage organs, such as rhizomes and tubers. It is very effective for the control of perennial weeds and is more efficacious than many other nonselective herbicides that only affect the aboveground parts of the plant. Applied to soil, glyphosate shows little activity because the strong binding to soil organic matter makes it biologically unavailable for uptake by plants. Glyphosate has been used extensively in Colombia and many other countries for agricultural and other purposes for many years. Use of glyphosate in the coca and poppy spray program is shown in Table 1 and represents a relatively small fraction of the total use in Colombia.

Table 1. Glyphosate use in Eradication Spraying in Colombia, 2000–2004

Year	Amount sold in Colombia (L)[a]	Amount used in the eradication of illicit crops (L)[b]	Percent of total amount sold
2000	7,037,500	603,970	8.6%
2001	9,473,570	984,848	10.4%
2002	NA	1,061,538	11%[c]
2003		1,381,296	14%[c]
2004		1,420,130	14%[c]

[a] Data from ICA (2003).

[b] Data from Dirección Nacional de Estupefacientes (2002); Policia Nacional Dirección Antinarcóticos (2005).

[c] Estimated from total used in 2001 but likely less than this value.

Environmental Fate. The environmental fate of glyphosate has been extensively reviewed (CWQG 1999; Giesy et al. 2000; NRA 1996; World Health Organization International Program on Chemical Safety 1994); only key issues relevant to water and soil/sediment are summarized next.

As a result of its specific physicochemical properties, glyphosate is immobile or only slightly mobile in soil. The metabolite of glyphosate, aminomethyl phosphoric acid (AMPA; see Fig. 1), is somewhat more mobile in soil but is rapidly broken down, resulting in minimal amounts leaching in normal agricultural soils. The strong binding of glyphosate to soil results in almost immediate loss of biological activity; however, the bound residues do break down sufficiently rapidly that accumulation will not occur, even over many years of regular use. Contamination of groundwater from the normal use of glyphosate is unlikely except in the event of a substantial spill or other accidental and uncontrolled release of large amounts into the environment.

The high water solubility of glyphosate and its salts suggests that it would be mobile in water; however, strong and rapid binding to sediments and soil particles, especially in shallow, turbulent waters, or those carrying large loads of particulates, removes glyphosate from the water column (Tooby 1985). In normal agricultural uses, it is not expected to run off or leach into surface waters.

In water, the two major pathways of dissipation are microbiological breakdown and binding to sediments (Giesy et al. 2000; World Health Organization International Program on Chemical Safety 1994). Glyphosate does not degrade rapidly in sterile water, but in the presence of microflora (bacteria and fungi) in water, glyphosate is broken down to AMPA (see Fig. 1) and eventually to carbon dioxide (Rueppel et al. 1977). Other metabolic pathways have been reported (Liu et al. 1991), including further degradation of AMPA to inorganic phosphate and CH_3-NH_3, and via sarcosine to glycine (see Fig. 1). None of these products is considered herbicidal and would not be expected to be highly toxic to aquatic organisms at concentrations that would result from field use of glyphosate in aquatic systems. Photodegradation also may take place under field conditions where sufficient penetration of UV radiation occurs.

The dissipation of glyphosate from treated foliage and from leaf litter has also been characterized. As would be expected, most of the glyphosate sprayed on the plants penetrates into plant tissues after application, but some is available for washoff for several days after application (World Health Organization International Program on Chemical Safety 1994). If the plant dies as a result of this exposure, glyphosate would be present in the dead and decaying plant tissues. Glyphosate residues in leaf litter dissipate rapidly with a time-to-50%-disappearance (DT_{50}) of 8–9 d under temperate forestry conditions (Feng and Thompson 1990). Similar rapid dissipation from fruits and lichen has also been observed (Stiltanen et al. 1981) in north temperate regions.

Under tropical conditions such as in Colombia, dissipation will likely be more rapid than in temperate regions because of higher temperatures and moisture content, which promote microbiological activity as well as chemical degradation of many pesticides. Large areas of Brazil, Colombia, and Central America share similar tropical conditions and depend heavily on herbicides such as glyphosate (Racke et al. 1997). Glyphosate has been used in large areas of Brazil on no-tillage crops in general and, more recently, on transgenic soybeans. Comparing the fate of pesticides in tropical and temperate conditions, Racke et al. (1997) found no evidence of particular behavior of pesticides in the tropics and concluded a greater rate of degradation occurs under tropical conditions.

Formulants and Adjuvants

Formulants are substances that are added to a pesticide active ingredient at the time of manufacture to improve its efficacy and ease of use. These formulants serve many purposes and comprise a large range of substances, from solvents to surfactants to modifiers of pH. The glyphosate formulation used in Colombia includes several formulants. Adjuvants are added to formulated pesticides at the time of application and, like formulants, increase efficacy, or ease of use in special situations where pests are difficult to control or where nontarget effects need to be minimized. In the control program for illicit crops in Colombia, an adjuvant, Cosmo-Flux, is added at the time of spraying.

The relatively large water solubility and the ionic nature of glyphosate retard penetration through plant hydrophobic cuticular waxes. For this reason, glyphosate is commonly formulated with surfactants that decrease the surface tension of the solution and increase penetration into the tissues of the plants (Giesy et al. 2000; World Health Organization International Program on Chemical Safety 1994).

Surfactants in the Glyphosate Formulation. The glyphosate formulation as used in eradication spraying in Colombia contains formulants that are common to the commercial product as used in agriculture. Cosmo-Flux 411F, an agricultural adjuvant containing nonionic surfactants (a mixture of linear and aryl polyethoxylates, 17% w/v) and isoparaffins (83% v/v) (Cosmoagro 2004) is added to the spray solution. Adjuvants such as these are commonly added to pesticide formulations to improve efficacy through several mechanisms (Reeves 1992; Tadros 1994).

For example, surfactants such as the polyethoxylates in Cosmo-Flux, increase efficacy through increasing target surface adherence, promoting better droplet spread, better dispersion, prevention of aggregation, and enhanced penetration of herbicides into target plant tissues through the reduction of surface tension on plants. Surfactants can also disrupt the hydrophobic wax cuticle, thus increasing the penetration of active ingredient.

Base oils, such as the isoparaffins in Cosmo-Flux, are another class of adjuvants used in formulations. They are used primarily to aid foliar absorption of the pesticide by disrupting the waxy cuticle on the outer surface of foliage, which increases cell membrane permeability (Manthey and Nalewaja 1992).

Coca and Poppy Control Programs

The coca and poppy control programs make use of several procedures to identify, locate, and map coca and poppy fields. The initial step in this process is the use of satellite images to locate the fields. These images are provided by North American and European governments to the Government of Colombia. The images are used to locate potential areas of production. Further visual observations are made using overflights with observers and/or photographs from a low-altitude aerial photography aircraft, such as a Cessna Caravan, to verify the presence of coca and poppy fields. The camera used for this purpose is multispectral high resolution. Maps are generated in a Geographic Information System (GIS) and are used to produce updated coordinates for the spray pilots, as well as information for downloading into the aircraft navigation systems (Policia Nacional Dirección Antinarcóticos 2005). Field operation offices for the control program have computers and a satellite uplink for data transfer. Spray planes are equipped with high-resolution tracking equipment and Del Norte positional data recorders that display position, provide directional guidance, and store positional data and spray information on data cards for later analysis. Thus, field locations, flight paths of the spray planes, and areas where spray is released are known to within a resolution of 1–2 m.

Since 1994, coca and, more recently, poppy fields have been identified and sprayed. Total areas of identified fields and the area sprayed in Colombia are shown in Fig. 2. With increasing areas sprayed, the total area planted to coca has generally decreased since 2000.

Receiving Environment. Colombia is located between 4° S and 12° N of the equator. The country presents varied topography ranging from snow-capped peaks through high mountain plateaus to low-lying tropical regions. In general, coca tends to be grown at altitudes below 1,500 m and poppy at greater altitudes, usually 2,200 m. The biodiversity hotspot for the tropical Andean region includes significant areas of Colombia (Fig. 3). The tropical Andes biodiversity region is estimated to contain 15%–17% of the world's plant life in only 0.8% of its area. It has an area of 1,258,000 square kilometers, extends from Western Venezuela to Northern Chile and Argentina, and includes large portions of Colombia, Ecuador, Peru, and Bolivia (Centre for Biodiversity 2004).

Because the diversity hotspots are mainly associated with the Andean highlands and coca is mostly grown in lower altitudes, there is only some overlap between areas of coca production and regions of high biodiversity. Poppy is grown at a greater altitude, which overlaps with the biodiversity hotspot; however, the total areas grown at this time are small (see Fig. 2). Exact areas used for coca and poppy production within the diversity hotspot are not known; however, this information would be useful for assessing total impacts of production, especially for rare and endangered plant species.

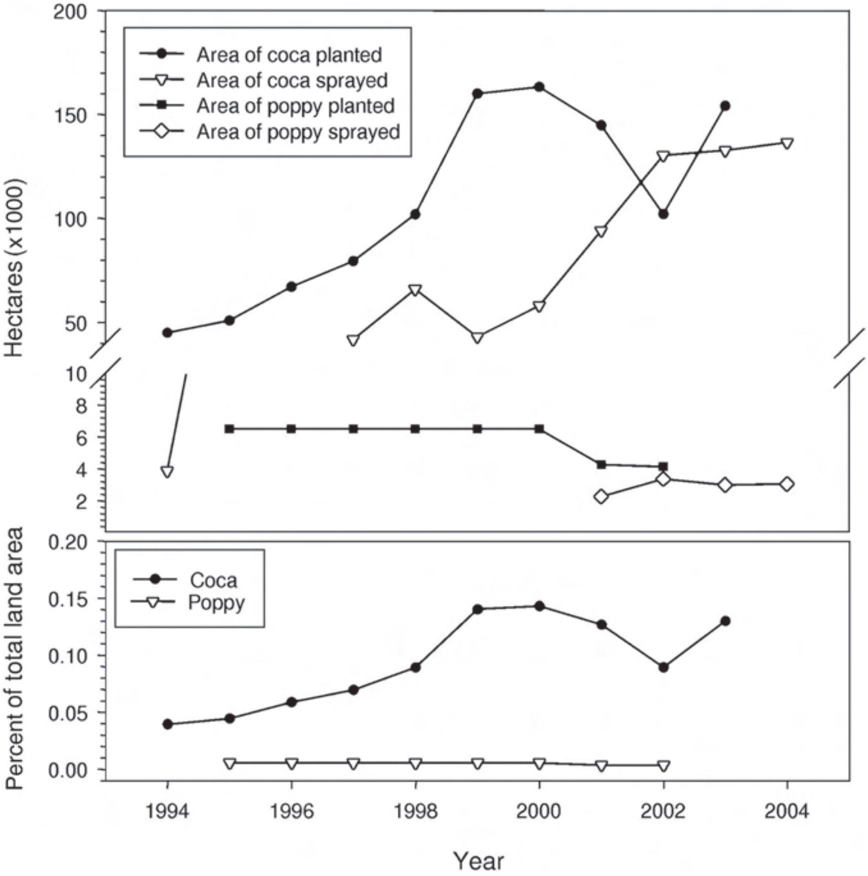


Fig. 2. Areas planted with coca and poppy in Colombia from 1994 to 2002 as hectares (ha) (*above*) and as a percent of the total land area of Colombia (*below*).

Method of Application. All coca and poppy fields are sprayed from fixed-wing aircraft. The procedure described below is based on observations recorded for the AT 65, AT 802, and OV 10 aircraft.

Spray planes are loaded in a special area of the tarmac (Fig. 4) at a number of bases throughout Colombia. Glyphosate and Cosmo-Flux are stored in plastic containers in a tarp-lined area protected by a berm to contain accidental spills. The areas may be in the open or covered. Glyphosate is transferred from 200-L plastic barrels to a larger plastic storage tank (Fig. 4A). Cosmo-Flux is transferred from 20-L plastic containers to a mixing tank. The required amounts of the components of the application mixture (glyphosate, Cosmo-Flux, and water from a local source) are pumped through a metering pump (Fig. 4B) into the aircraft using a table of mixing proportions to ensure the correct ratio of amounts are loaded.

Fig. 3. Map showing the region of Colombia identified as part of the Andean Biodiversity Region.

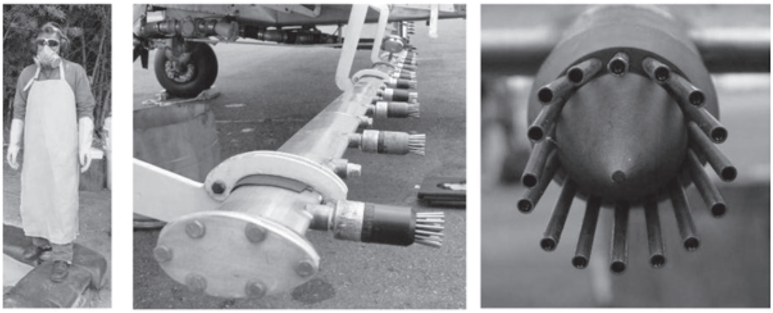
Appropriate protective equipment is used by the mixer-loaders, who are trained in the loading procedures (Fig. 4C).

The spray boom (Fig. 4D) on the aircraft is equipped with raindrop nozzles (Fig. 4E). These nozzles produce droplets with a volume mean diameter (VMD) of 300–1,500 µm and are similar to those used in forestry spraying for site preparation (Payne 1993). The aircraft spray systems are electronically calibrated to disperse a specified quantity of spray mix per hectare, compensating for variances in ground speed. These electronic spray controls are checked each day by technicians and also during the pilot's preflight inspection. During actual spray operations, the pilot monitors the spray system by reading the spray pressure and flow rate gauges (United States Department of State 2002).

The same nozzles are used for both coca and poppy applications, but twice as many are used for the poppy applications at different boom

A) Mixing area for glyphosate and adjuvants

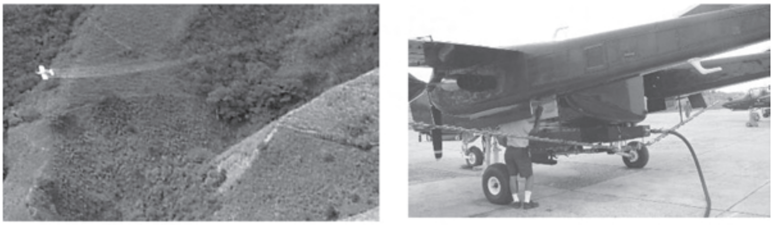
B) Mixer for glyphosate and adjuvant



C) Mixer-loader

D) Spray boom

E) Nozzle



F) AT-65 spray plane

G) OV10 Spray plane being loaded

H) Del Norte GPS system

I) Positional data

J) Spray locations

Fig. 4. Photographs of aspects of the spray operation.

Table 2. Application Rates of Glyphosate and Cosmo-Flux for Control of Coca and Poppy.

	L/ha		kg AE/ha[a]	
	Coca	Poppy	Coca	Poppy
Glyphosate	10.4	2.5	4.992	1.2
Cosmo-Flux	0.24	0.51		

[a]Glyphosate acid equivalent (AE)/ha. *Source*: Dirección Nacional de Estupefacientes (2002).

pressures. As a result, coca and poppy applications are done separately. Currently used application rates are shown in Table 2.

Each spray operation (Fig. 4F,G), which may consist of two or more spray planes, is escorted by search-and-rescue (SAR) helicopter(s) in case of an accident or incident. Spraying is only conducted in daylight hours before midafternoon to ensure that conditions are appropriate for application. If rain is imminent, visibility is poor, or wind speed is in excess of 7.5 km/hr (4 knots), spraying is not carried out. Wind speed is checked during the operation by the SAR and other helicopters with the aid of smoke generated by the spray planes. Spraying is done at about 30 m above ground and, although the flight path is determined from the GIS information and the Del Norte guidance system (Fig. 4H), the actual spraying is controlled by the pilots. In personal communications with five of the pilots, it was stated that, according to spraying guidelines, fields are not sprayed if people are or soon will be present.

After a spray operation, the flight path of the spray planes and the areas sprayed are downloaded from the Del Norte system (Fig. 4I) and processed by GIS to show the spray patterns and calculate the areas spayed (Fig. 4J). This information is transmitted to the DIRAN where records of the spray operations are retained and used for compilation of annual reports and statistics (Dirección Nacional de Estupefacientes 2002).

Frequency of Application. Frequency of application varies with local conditions and actions taken by the growers after the coca or poppy is sprayed. When coca is sprayed, some growers prune the bushes down to about 10 cm above ground in an attempt to prevent translocation of the herbicide to the roots. Sometimes these plants will recover and resprout; however, they will not yield large amounts of coca leaves for several months. If the field is replanted to coca from seedlings, reasonable productivity may not be achieved for 4–6 mon. If the field is replanted from cuttings, productivity may be achieved sooner. Thus, spraying of a particular coca field may have a return frequency of about 6–12 mon.

Being an annual, poppy is grown from seed. In the climatic conditions in Colombia, poppy fields would be harvested twice a year. If sprayed before reaching maturity and replanted immediately after spraying, they may be sprayed four times a year.

Exposure Pathways in Soil, Air, Water, and Other Media. In terms of application, there are several pathways through which glyphosate and adjuvants may contact the environment (Fig. 5).

Deposition on the target crop is the desired outcome; however, for purposes of assessing risks in humans and the environment, exposures that result in movement and deposition off the field are important. Spray drift would result in movement off target and could result in adverse effects in nontarget plants and animals. Given the strong adsorption of glyphosate to soil, deposition on soil in the field will likely not result in significant effects on nontarget organisms; however, runoff of residues bound to soil particles may result in contamination of surface waters with sediment-bound residues. Direct deposition and spray drift may result in contamination of local surface waters with glyphosate if these are in the spray swath or drift envelope of application. Depending on the depth of water, turbulence, flow, and presence of suspended particles, this would result in exposures of aquatic organisms to both glyphosate and any adjuvants present in the spray. Organisms present in the field during spraying would be exposed to the spray droplets and receive a theoretical dose, depending on surface area exposed and body mass.

Off-Target Deposition. There are two types of off-target deposition. The first is related to incorrect application where the spray pilot initiates application too soon or turns off the spray too late, or the spray swath includes a nontarget area on one or both sides of the target field. The second type

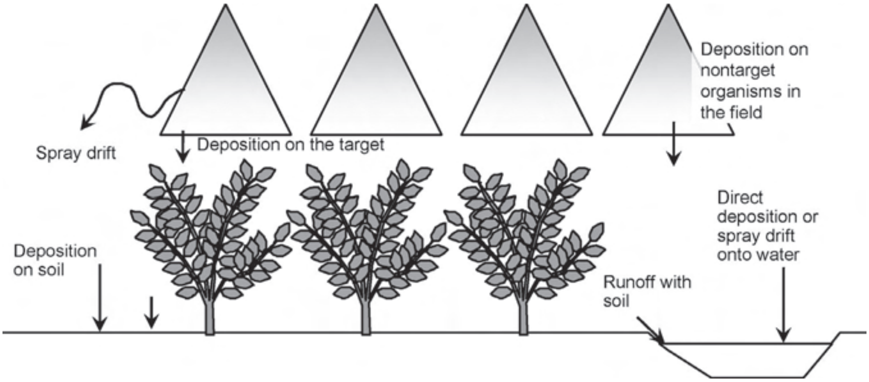


Fig. 5. Diagram showing exposure routes for various environmental compartments when glyphosate is used for the control of illicit crops.

of off-target deposition is spray drift. Experience with spray equipment of the type used in Colombia suggests that spray drift will be minimal (Payne et al. 1990). Estimates of accidental overspray have been made during assessments of spray program efficacy (Helling 2003). Based on site visits to 86 fields sprayed in 2002 and observations of damaged plants beyond the boundary of the area cleared and planted with coca, 22 fields showed evidence of off-field deposition. Using the size of these areas, it was estimated that between 0.25% and 0.48% of the areas cleared for coca production were damaged by offsite spray deposition (Helling 2003). Applying this estimate to the total area of coca sprayed (see Fig. 2) and calculating upper and lower intervals, the areas potentially affected are small when compared to the total area of Colombia (Table 3).

Although the areas affected by off-target drift are estimated to be small, this estimate is based on visual observations of a relatively small number of fields. These data were available only for coca, not poppy; however, the total areas planted to poppy are not large, and similar off-target deposition would be proportionately smaller than that associated with coca production. This lack of data is a source of uncertainty in the assessment. It is not logistically possible to visually inspect all sprayed fields; however, routine monitoring of the areas planted to coca and poppy that is undertaken by satellite and low-altitude imagery could be used to assess off-target deposition resulting in damage to plants. Changes in the size of sprayed fields over time could be used to extend these estimates over larger areas and increase their accuracy, although extension of the fields by growers may confound the data. The lower resolution of satellite imagery may preclude its use for this purpose; however, greater coverage by low-altitude images could facilitate this process.

Table 3. Estimates of Areas Affected by Off-Target Deposition of Glyphosate in the Spraying of Coca in Colombia.

Year	Hectares sprayed (ha)	Area affected by off-target deposits (ha)		Upper interval as percent (%) of the total area of Colombia
		Lower interval (0.25%)	Upper interval (0.48%)	
1994	3,871	9.7	18.6	0.0000002
1995	23,915	59.8	114.8	0.0000010
1997	41,861	104.7	200.9	0.0000018
1998	66,029	165.1	316.9	0.0000028
1999	43,111	107.8	206.9	0.0000018
2000	58,074	145.2	278.8	0.0000024
2001	94,152	235.4	451.9	0.0000040
2002	130,364	325.9	625.7	0.0000055
2003	132,817	332.0	637.5	0.0000056
2004	136,551	341.4	655.4	0.0000057

B. Framework for Risk Assessment

The following sections outline the conceptual model and hypotheses for the assessment of the human health and environmental impact of coca and poppy production in Colombia.

Conceptual Model

For purposes of the risk assessment of the use of glyphosate and adjuvants in the control of poppy and coca, the conceptual model applied was that normally applied to the agricultural application of pesticides where hazard and risk are directly related to toxicity and exposure. Thus, for human health, toxicity data were compared to exposures estimated from worst case data and also from more realistic data obtained in other uses of glyphosate, such as agriculture and forestry. Because of the low frequency of spray application, exposure from this source is acute and resulting risks were compared to acute toxicity data. Toxicity data for the active ingredient, glyphosate (IPA), were obtained from the literature and from the results of acute laboratory animal tests conducted with the mixture of formulated glyphosate and Cosmo-Flux as used in the spray program in Colombia. It is possible that glyphosate used in the eradication program may contribute to exposures via the food chain and drinking water; these exposures were estimated and compared with toxicity data and exposure guidelines based on chronic toxicity for glyphosate via dietary exposures. In addition, specific human health responses were assessed in epidemiological studies conducted in Colombia.

In assessing ecological risks, a similar agriculture-based approach was used. Similar to the foregoing approach, exposures were estimated from worst case models, from measurements made in other locations, and from measurements based on samples collected from the environment in Colombia. Because of the long periods between applications, ecological exposures from the spray operations are acute and were compared to acute toxicity data. Toxicity data were obtained from the literature and from laboratory-based tests on standard test organisms that were specifically conducted on the spray mixture as used in Colombia. The risk hypotheses are discussed next, and the remainder of this review focuses on tests of these hypotheses.

Risk Hypotheses

A large number of hypotheses were actually tested in this risk assessment; however, they were basically the same hypothesis with minor differences in the exposure and toxicity parameters. As is normal in the scientific method (Popper 1979), these hypotheses are stated as the null or negative hypothesis; thus, we attempted to falsify or disprove these hypotheses through the use of appropriate data.

For human health, two main hypotheses were used:

- Exposures to glyphosate and adjuvants as used in the poppy and coca eradication programs do not cause acute adverse effects to humans exposed via a number of routes.
- The use of glyphosate and adjuvants in those locations where eradication of poppy and coca are conducted does not result in acute and chronic health outcomes that are different from other locations where glyphosate is not used or is used in other agricultural practices.

For ecological effects, one main hypothesis was used:

- Exposures to glyphosate and adjuvants as used in the poppy and coca eradication programs do not cause acute adverse outcomes on nontarget organisms exposed via a number of routes.

III. Exposure Characterization

Exposure characterization is one of the key components of any risk assessment (NRC 1993; USEPA 1992, 1998). No measurements of farmer or pesticide applicator exposures have been made in Colombia. An assessment of pesticide use among farmers in the Amazon Basin of Ecuador has shown that paraquat and glyphosate are widely used. Risk behaviors were identified as frequent pesticide use, washing pesticide equipment in water sources used by humans, inadequate disposal of empty containers, eating and drinking during application, and using inadequate protective clothing (Hurtig et al. 2003). However, agricultural uses such as these are quite different from the aerial applications in Colombia. In the following sections, the potential for exposures in humans and the environment to glyphosate as used in the eradication program is discussed and characterized.

Human Exposure

In the agricultural setting, two groups are usually considered—applicators and bystanders. The group that experiences the greatest probability of exposure is the applicator group, which here includes mixer-loaders, spray plane pilots, and technicians who service the aircraft. The second group includes bystanders who may come into contact with the herbicide during application via direct deposition if they are within the spray swath, are directly exposed to spray drift, are exposed to deposits of spray when they reenter treated fields, or are exposed through the consumption of sprayed food items or contamined drinking water.

Applicator Exposure. Risk to applicators was not a specific target of this assessment; however, their exposure can be characterized. Based on observations of spray operations in several locations in Colombia, a number of measures are taken to reduce potential applicator exposure (Table 4).

Table 4. Protective measures used to reduce exposure of applicators to glyphosate and formulants as used in poppy and coca eradication programs.

Applicator subgroup	Mixer-loader	Spray pilot	Aircraft technician
Technology for handling of the formulation and spray mix	Use of closed-loading systems and pumps to mix and transfer glyphosate and Cosmo-Flux to the aircraft.	Not involved in mixing and loading	Not normally involved in mixing and loading; aircraft are washed down regularly so that exposure via contaminated surfaces is reduced
Protective equipment worn	Long pants, long sleeves, full rubber apron, rubber gloves, cloth hat or cap, particulate air filter and dark glasses, leather military-style boots	None other than normal clothing, long sleeves, long pants, jacket, and boots	Short or long sleeves, shorts or long pants, boots or sneakers, cloth cap or none
Equipment used to remove contamination, should it occur	Eye-wash station at all locations, clean water for washing hands and any contaminated surfaces, a shower in some locations	Same as is available to the mixer-loader	Same as is available to the mixer-loader

No measures of exposure were available for mixer-loaders in Colombia; however, they are likely to be similar to those of applicators in other situations. Based on observations on forestry and agricultural applicators (Acquavella et al. 2004; and summarized in Williams et al. 2000), exposures are generally small. Peak estimated exposure in applicators from all routes was 0.056 mg/kg body weight (bw). The estimate of chronic exposure from all routes was 0.0085 mg/kg/d based on an 8-hr day and a 5-d work week. In the results of the recently published Farm Family Exposure Study, the greatest estimated systemic dose in a sample of 48 applicators was 0.004 mg/kg bw (Acquavella et al. 2004). In Colombia, mixing and loading are done by one or two individuals wearing appropriate protective equipment. Pilots have limited opportunity for exposure and, as has been observed in other studies (Frank et al. 1985), likely experience less exposure.

Exposures of mixer-loaders in Colombia are likely to be similar to those observed in agricultural applications. Exposures for spray pilots and technicians will likely also be less than for applicators. While most of the protective clothing worn by mixer-loaders is appropriate, the need for a respirator is questionable and the use of dark glasses in place of a full face shield is judged inappropriate. Dark glasses will not protect the eyes from a splash to the forehead that runs into the eyes, a vulnerable area in terms of glyphosate exposure during mixing and loading (Acquavella et al. 1999). A full face shield offers better protection. As glyphosate is not volatile, nor atomized during mixing and loading, use of a respirator offers little reduction in potential exposure and complicates the use of a full face shield. The usefulness of a respirator is judged to be small.

Bystander Exposure. Bystanders can be classified into several classes, depending on their route of exposure. These are discussed in the following sections.

Bystanders Directly Oversprayed. Although it is unusual for people to be present in a coca field during application, it is possible that a person could be standing directly in the spray swath and would receive a direct application of the spray. Several scenarios could occur (Fig. 6, Table 5). The most likely is the partially clothed human with a cross-sectional area of 0.25 m^2 exposed to the spray (Table 5). Given that glyphosate penetrates poorly through the skin with maximum penetration of about 2% (Williams et al. 2000), the body dose under a reasonable worst case exposure will be approximately 0.08 mg/kg bw.

Bystander exposure to glyphosate was estimated as 0.0044 mg/kg bw/d for a child 1–6 yr of age (Williams et al. 2000). Exposures to glyphosate were measured in bystanders to farm applications (Acquavella et al. 2004). These studies were conducted in spouses and children not involved in applications, and frequency of measurable exposure was small, with 4% and 12% of the spouses and children, respectively, with detectable exposures based on

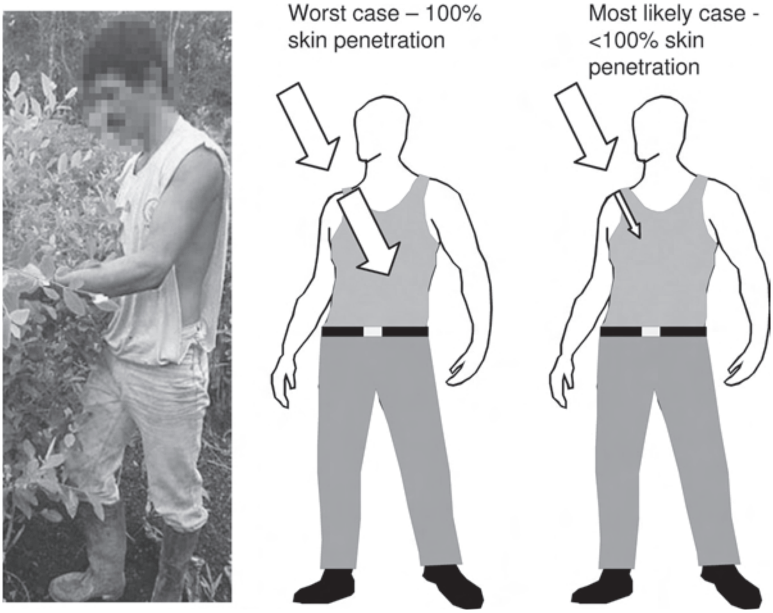


Fig. 6. Illustration of human exposure scenarios.

Table 5. Estimates of human exposure to glyphosate during a spray application.

	Exposure in mg/kg bw	
Scenario	Coca: 4.992 kg/ha	Poppy: 1.2 kg/ha
Partially clothed human with cross-sectional area of $0.25\,m^2$, complete penetration	1.8	0.4
Partially clothed human with cross-sectional area of $0.25\,m^2$, 2% penetration (most likely)	0.04	0.01

Assumptions: body weight, 70 kg; body surface area, $2\,m^2$.

urinary monitoring. The maximum systemic dose estimates for spouses and children were 0.00004 mg/kg and 0.0008 mg/kg bw, respectively (Acquavella et al. 2004). If bystanders are neither directly sprayed nor reenter the field immediately after spraying, their exposures will likely be within a factor of 10 of farm bystanders. All these measured exposures are considerably less than those estimated in Table 6, considered to be reasonable worst case values.

Reentry. If a person were to reenter the sprayed field immediately after spraying and come into contact with the treated foliage, such as when

Table 6. Estimates of human exposure to glyphosate during reentry to treated fields.

	Exposure in mg/kg bw	
Scenario	Coca: 4.992 kg/ha	Poppy: 1.2 kg/ha
Maximum reentry exposure estimated for an adult human with a 10-hr d	0.013	0.003
Maximum reentry exposure estimated for a 1- to 6-yr-old child with a 10-hr d	0.259	0.062

attempting to pick leaves from sprayed coca plants, exposure to glyphosate could occur through the hands and arms. Given the area exposed, the small penetration, and the saturation of the transfer that would result once the hands were wet, total body dose is likely to be less than the reasonable worst case scenario described in Table 6. The potential for reentry exposure has been summarized by Williams et al. (2000). Reentry exposures decreased with time after application and, on day 7 after application, were 3% of those estimated for day 1. Reentry into areas of tall weeds (height, 1.5 m) resulted in 10-fold-greater exposures than in areas of short grass. Based on measurements in farmworkers, estimates of reentry exposure to glyphosate in adults ranged from 0.0000039 to 0.0026 mg/kg bw/hr of reentry time. Maximum reentry exposure for a 1- to 6-yr-old child was estimated at 0.026 mg/kg bw for a 5-hr contact period. As these estimates are based on a spray application rate of 1 kg/ha, reentry exposures under Colombian conditions are estimated to be somewhat greater (see Table 6). These numbers are also greater than the direct overspray, as the persons involved may have repeated exposures if they reenter a field immediately after spraying.

Inhalation. Because the vapor pressure of glyphosate (isopropyl ammonium salt) is low (2.1×10^{-3} mPa at 25°C) and it also has a small Henry's law constant (4.6×10^{-10} Pa m^3 mol^{-1}) (BCPC 2003), it will not be present in air as a vapor at biologically relevant concentrations. The droplet sizes resulting from the spray application of glyphosate in Colombia are large, with a mean droplet diameter ~1,000 µm and with very few droplets <500 µm. As such, they are unlikely to be inhaled and penetrate into the lungs. Based on measurements of glyphosate concentrations in air during applications, the maximum estimated daily dose (8 hr) resulting from inhalation of spray droplets by applicators was 0.0062 mg/kg bw (Williams et al. 2000), a value that is judged to be applicable as a maximum exposure for bystanders.

Dietary and Drinking Water. Dietary and drinking water exposures to glyphosate have been estimated to be relatively small under conditions of use in North America (Williams et al. 2000) (Table 7).

Table 7. Worst case daily human exposure estimates for glyphosate (mg/kg bw/d)

	Female adult		Female child (1–6 yr)	
Sources	Acute	Chronic	Acute	Chronic
Drinking water	0.000036	0.000002	0.000110	0.000004
Diet	0.024	0.024	0.052	0.052
Wild foods	0.045		0.045	
Total from diet and water	0.069	0.024	0.097	0.052
Values extrapolated from the above (Williams et al. 2000) to the greater application rate of 4.992 kg/ha used in control of coca				
Drinking water	0.000179	0.00001	0.00055	0.000018
Diet	0.119	0.119	0.259	0.259
Wild foods	0.224	0.224	0.224	0.489
Total from diet and water	0.343	0.293	0.483	0.747

The results of monitoring programs conducted by the Danish Veterinary and Food Administration from 1997 to 1999 reported on the content of glyphosate and several other pesticides in cereals produced in Denmark (Granby and Vahl 2001). Based on the residues of glyphosate in cereals, intake of glyphosate for a 60-kg adult was estimated at 0.007 mg/d.

Based on a study of 51 streams in nine midwestern U.S. states, the U.S. Geological Survey (USGS) reported the presence of glyphosate and a number of other herbicides in surface waters (Battaglin et al. 2005; Scribner et al. 2003). Of a total of 154 water samples collected during 2002, glyphosate was detected in 36%, and its degradation product, aminomethylphosphonic acid (AMPA), was detected in 69%. The greatest measured concentration of glyphosate in any sample was 8.7 µg/L, and the greatest concentration of AMPA detected in the USGS study was 3.6 µg/L. More recently, glyphosate and AMPA have been detected in association with urban inputs from wastewater treatment in the U.S. Maximum concentrations of glyphosate and AMPA were 2 and 4 µg/L, respectively (Kolpin et al. 2006). Concentrations of glyphosate detected in Colombian surface waters (see following) were usually less than 25 µg/L, the detection limit. Exposures from drinking untreated surface waters in areas where spraying takes place are judged to be small and infrequent.

Environmental Exposures

Air. The presence of glyphosate in air is unlikely because it, and the salt forms commonly used in glyphosate formulations, have essentially negligible vapor pressure. Spray droplets may, however, be present in air and are the likely reason for detection of glyphosate, along with other pesticides, in rainwater in the European Union (EU) (Quaghebeur et al. 2004). From 1997 to 2001, glyphosate was only detected in rainwater in Belgium in 2001 and then with a frequency of 10% and a maximum concentration of 6.2 μg/L.

Water. If water is directly oversprayed, contamination of surface waters will result (see Fig. 5). Some coca fields are located near ponds and lakes and some are near streams and rivers (Helling 2003). Although surface waters are not deliberately sprayed, some overspray of small watercourses and the edges of ponds, reservoirs, and lakes may occur. In the absence of measured concentrations immediately after spraying in surface waters located close to fields, estimates of exposure were made using worst case assumptions (Table 8) based on water depth assumptions used by the USEPA (Urban and Cook 1986) and the EU (Riley et al. 1991).

Glyphosate has been detected in surface waters (see foregoing discussion on human exposures through drinking water) in a number of locations.

Table 8. Estimates of concentrations of glyphosate in surface water after a spray application.

	Exposure in μg/L (glyphosate[a])	
Scenario	Coca: 4.992 kg/ha (3.69 kg AE/ha)	Poppy: 1.2 kg/ha (0.89 kg AE/ha)
Surface water, 2 m deep, rapid mixing and no absorption to sediments, no flow	185	44
Surface water, 0.3 m deep, rapid mixing and no absorption to sediments, no flow	1,229	296
Surface water, 0.15 m deep, rapid mixing and no absorption to sediments, no flow	2,473	595
Surface water, 0.15 m deep, rapid mixing and 50% absorption to sediments, no flow	1,237	297

[a] Note that the concentration is expressed as glyphosate acid (AE) to allow comparison to exposures used in environmental toxicity testing. In both these exposures and in the toxicity testing of Cosmo-Flux, proportional amounts are present and the exposure and toxicity values are thus directly comparable and can be used to assess the hazard of the mixture as applied in Colombia.

Glyphosate residues have been reported in surface waters in Denmark as a result of agricultural activities. These residues were observed as part of the Pesticide Leaching Assessment Program (PLAP), a project that was intended to study leaching potential of pesticides to groundwater (Kjaer et al. 2003, 2005). PLAP was focused on pesticides used in farming and monitored leaching at six agricultural test sites representative of Danish conditions. Water from special drilled wells and from normal tile drains was analyzed for glyphosate and aminomethylphosphonic acid (AMPA, a major degradate of glyphosate). It is not clear from the report if the samples were filtered before analysis; this is important as glyphosate binds strongly to organic matter in soils and can be transported in this form. The presence of macropores in soil would facilitate transport to the tile drains.

In the samples from PLAP collected following glyphosate applications, there were no detections of glyphosate or AMPA that exceeded 0.1 μg/L in any of the groundwater samples taken from suction cells (1 and 2 m below the surface), the vertical wells (about 1.5–5.5 m below surface), and the horizontal wells (about 3.5 m below surface).

Glyphosate residues were detected in water from tiles draining the field and were observed primarily in the autumn. The highest measured concentrations were 5.1 μg/L for glyphosate and 5.4 μg/L for AMPA. The calculated average annual concentrations of glyphosate and AMPA in drainage water were 0.54 and 0.17 μg/L, respectively, at one location, and 0.12 and 0.06 μg/L, respectively, at a second. At a third location, glyphosate and AMPA were detected but average concentrations of both were below 0.1 μg/L. In Danish soils, degradation of glyphosate was shown to be slower in sandy soils than gravel but leaching was observed only in rounded gravel soils (Strange-Hansen et al. 2004), and leachate concentrations were less than 0.1 μg/L (Fomsgaard et al. 2003). Similarly, a study on fate of glyphosate in soils showed rapid dissipation with almost total dissipation 1 mon after application (Veiga et al. 2001). Given the small organic content of gravel and the presence of macropores between the grains of gravel, movement through this matrix is not surprising. Complete degradation in other types of soil is expected.

Other authors have reported glyphosate residues in surface waters in Europe (Skark et al. 1998, 2004), although the frequency of detection was not large. These authors suggested that the contamination was from application to railroad beds, environments where gravel is used and where adsorption would be expected to be minimal. This conclusion is supported by other studies on the dissipation of herbicides applied to railroad beds (Ramwell et al. 2004) and highways (Huang et al. 2004; Ramwell et al. 2002). Application of glyphosate to hard surfaces in an urban context (road edges) can give peak runoff concentrations of 650 μg/L (Ramwell et al. 2002), but only 15 μg/L from a railway trackbed (Ramwell et al. 2004). In Germany, a study of two catchments found that nonagricultural pesticide use contributed more than two-thirds of the whole observed pesticide load in the tributaries and at least one-third in the Ruhr River (Skark et al. 2004). Most nonagricultural pesticides were derived from runoff from domestic, industrial and railway areas. Nevertheless, in Argentina, where glyphosate-tolerant soybean is now extensively grown and regularly treated, no residues have been observed in soil or water of either glyphosate or AMPA (Arregui et al. 2004).

The USGS study on midwestern U.S. streams (Battaglin et al. 2005; Scribner et al. 2003) analyzed water samples filtered through a 0.7-μm filter; thus, the concentrations represent dissolved glyphosate and AMPA. Measured values in this study ranged up to 8.7 μg/L.

Although the glyphosate concentrations in surface waters in other areas where it is used in agricultural and other activities are relatively small, concentrations have not been measured in Colombia. To address this uncertainty, we conducted a monitoring study to measure levels of glyphosate, AMPA, and other pesticides in surface waters.

This study was conducted in five locations in Colombia representing areas where spraying of coca was planned or where other agricultural activities were undertaken and were also near the human health studies. Sites were selected for safe access as well as ease of repeated sampling and are summarized in Table 9 with further details of temperatures, rainfall, and soil characteristics from separate reports (PTG 2005a–e).

To characterize concentrations of glyphosate and AMPA in surface waters, samples were taken weekly for 24 wk (CICAD/OAS 2004a). Samples, in plastic bottles, were frozen and held at −17°C until shipped to Canada for analysis using published methods (Thompson et al. 2004). The method detection limit (MDL) for the analysis was 25 μg/L. Duplicate samples were taken and one sample held in Colombia until the duplicate had been analyzed. In addition, field-spiked samples and blanks were taken at biweekly intervals. In addition to water, sediment samples were taken at monthly intervals for analysis of glyphosate and AMPA if significant concentrations were detected in surface waters. Appropriate field spikes and blanks of sediment were also taken bimonthly. Quality control samples showed excellent recovery and precision of the analytical method with 98% recovery for glyphosate and 8.8% coefficient of variation (CV), and 110% recovery efficiency for AMPA with 20% CV. Blank field sample analyses show no coextractive interferences above the MDL for either glyphosate or AMPA at any of the sample sites. Field-spiked samples showed no significant degradation of glyphosate during handling and transport with overall average value of 90% of expected concentrations.

Results are summarized in Table 9. In all locations and on most occasions, residues of glyphosate and AMPA were present at concentrations below the MDL of 25 μg/L. On one occasion each in Valle del Cauca and Boyacá, glyphosate concentrations of 30.1 and 25.5 μg/L, respectively, were found.

Table 9. Characteristics of sampling sites for glyphosate and other pesticides in surface waters and sediments in regions of Colombia and measured values in samples collected between October 2004 and March 2005.

Site name	Location	Altitude (m)	Major crop types	Known pesticide use	Frequency of detection of glyphosate (*n* of 24, %)	Other pesticides detected (*n*/*N*) and types
Valle del Cauca, Río Bolo	3°27.642′ N 76°19.860′ W	1002	Sugar cane	Glyphosate and other pesticides	1 (4%)	(3/10) 2,4-D
Boyacá, Quebrada Paunera	5°40.369′ N 74°00.986′ W	557	Coca	Manual eradication, no aerial spraying of glyphosate	1 (4%)	(0/8)
Sierra Nevada, Quebrada La Otra	11°13.991′ N 74°01.588′ W	407	Organic coffee	None	0 (0%)	(0/9)
Putumayo, Río Mansoya	0°43.259′ N 76°05.634′ W	329	Coca	Aerial eradication spraying	0 (0%)	(0/9)
Nariño, Rio Sabaletas	1°27.915′ N 78°38.975′ W	15	Coca	Aerial eradication spraying	0 (0%)	(1/8) endosulfan I, endosulfan II, endosulfan sulfate

At these sites, spraying was not carried out and the only use of glyphosate, if any, was in agriculture. These data suggest that, at the watershed level, little or no contamination of surface waters with glyphosate at significant concentrations has resulted from the use of glyphosate in either agricultural or eradication spraying in Colombia. As concentrations in surface waters were mostly below the MDL, sediment analyses were not performed.

To characterize concentrations of other pesticides in surface waters and sediments, samples of water were taken in glass bottles every 2 wk for 22 wk (CICAD/OAS 2004b). Samples were held at 4°C until shipment to Canada for analysis. Analyses were conducted at the Laboratory Services Division of the University of Guelph using standard methods (LSD 2005). Duplicate samples were held in Colombia until analyses were completed. Field spikes and blanks were taken at 5-wk intervals, as were sediment samples. Sediment blanks and spikes were taken only once. These results are also summarized in Table 9. Blanks showed no contamination of samples during storage and shipping. Spiked samples showed variable recovery, particularly for the carbamate, carbaryl. Several pesticides were detected in surface waters, which is not unexpected as pesticides are widely used in agriculture in Colombia and, based on experience in other locations, some contamination of surface waters will occur. Of interest is the detection of endosulfan (I and II) and its breakdown product, endosulfan sulfate, in samples taken at the Nariño site. Endosulfan is not registered for use in Colombia, and its detection here likely is the result of illegal use. Whether this contamination resulted from regular agricultural activity or from use in the production of coca is unknown.

Soil. Concentrations of glyphosate in the top 25 mm of soil were estimated from the application rates and ranged from 1.6 to 3.2 mg/kg for poppy use rates and from 6.7 to 13.3 mg/kg for coca, depending on assumptions about interception by the crop foliage (50%) and soil density (1.5 kg/L). Measurements could be made through the use of residue analysis; however, the more important question is the biological availability of the glyphosate, as this would determine its potential for biological effects.

Although there are no direct measurements of glyphosate and AMPA concentrations available from treated coca and poppy fields in Colombia, the biological activity of any residues that may be present is judged to be small as the sprayed fields rapidly become colonized with invasive plants or are replanted to coca soon after spraying. From visual observations (Fig. 7), from observation in other uses and other locations (above), and from other reports (Helling 2003), recolonization is rapid and there have been no adverse effects observed in terms of recolonization or replanting of sprayed fields.

Fig. 7. Photograph of coca plants near Caucasia, Colombia, replanted from cuttings in a field sprayed with glyphosate 56 days previously.

IV. Effects Characterization

A. Glyphosate

Human health and environmental effects of glyphosate have been extensively reviewed (Giesy et al. 2000; Solomon and Thompson 2003; Williams et al. 2000) and by regulatory agencies (NRA 1996; USEPA 1993a, 1997, 1999; World Health Organization International Program on Chemical Safety 1994). The following sections are primarily directed to a critical analysis of original articles published since 1999 or that were not included in the earlier reviews (Giesy et al. 2000; Solomon and Thompson 2003; Williams et al. 2000). In characterizing the effects of glyphosate, it is important to distinguish between glyphosate as the active ingredient (usually glyphosate IPA salt) and the formulated product, such as Roundup. Glyphosate salts readily dissociate into the free acid, and the acid and salts are considered toxicologically equivalent. Formulations of glyphosate contain additional formulants that modify uptake of the glyphosate into plants and may alter toxicity of the mixture. In the following sections, tests conducted with the active ingredient only are referred to as "glyphosate." Those tests where a formulation was used are referred to by the specific product name, or where this is not known, as "glyphosate formulation." To allow easy comparison between technical product and formulations, where possible, concentrations of glyphosate have been normalized to acid equivalents (AE).

Effects of Glyphosate on Mammals

Laboratory Toxicity Studies. The toxicity of glyphosate and the formulation Roundup were reviewed by Williams et al. (2000). Glyphosate acid and its isopropylamine salt have little acute toxicity by the oral, dermal, and subcutaneous routes of exposure (Table 10).

Toxicity was greatest by intraperitoneal administration. When rats and mice were given glyphosate orally or intraperitoneally, several stress symptoms, such as increased respiration, elevated rectal temperatures, and occasional asphyxial convulsions, were noted. Median lethal doses of 4,704 mg/kg bw to the rat and 1,581 mg/kg bw to the mouse orally were significantly higher than 235 and 130 mg/kg bw, respectively, median lethal doses obtained when glyphosate was given intraperitoneally. Lung hyperemia was the major lesion noted in the glyphosate-poisoned animal (Bababurmi et al. 1978).

There is limited information on acute toxicity in dogs. However, there is a retrospective study conducted of 482 glyphosate-related calls recorded at the Centre National d'Informations Toxicologiques Veterinaires (CNITV) of France between 1991 and 1994. Only 31 cases were assessed as certain or highly probable and were linked with direct ingestion of

Table 10. Acute toxicity of glyphosate and formulations in selected mammals.

Species	Route	Compound administered[a]	LD_{50} (mg/kg bw)
Mouse	Oral	Glyphosate	>10,000
		Glyphosate	1,538
	Subcutaneous	Glyphosate saline	6,250 (M)
		Glyphosate saline	7,810 (F)
	Intraperitoneal	Glyphosate saline	545 (M)
		Glyphosate saline	740 (F)
		Glyphosate	134
Rat	Oral	Glyphosate, Roundup, glyphosate isopropylamine salt	>5,000
	Dermal	Roundup	>17,000
	Inhalation	Roundup, glyphosate saline	LC_{50} = 3.18 mg/L (4 hr)
	Subcutaneous	Glyphosate saline	17,500
		Glyphosate saline	281 (M) 467 (F)
	Intraperitoneal	Glyphosate	238
Rabbit	Oral	Glyphosate	3,800
	Dermal	Glyphosate, Roundup, glyphosate isopropylamine salt	>5,000
Goat	Oral	Glyphosate, Roundup, glyphosate isopropylamine salt	>3,500

Source: Smith and Oehme (1992).

glyphosate concentrates or spray in 25 dogs. The symptoms were most frequently described as vomiting, hypersalivation, and diarrhea; prostration and paresis were not common. Symptomatic treatment resulted in rapid recovery without sequelae (Burgat et al. 1998). Campbell and Chapman (2000) described the onset of clinical effects in dogs observed in several cases of poisoning as usually between 30 min and 2 hr. Recovery usually occurs over 1–2 d. Salivation, vomiting, diarrhea, irritation, and swelling of lips are common early features. Tachycardia and excitability are often present in the early stages, with the animals subsequently becoming ataxic, depressed, and bradycardic. Inappetence, pharyngitis, pyrexia, twitching, shaking, and dilated pupils are noted occasionally. Rarely, jaundice, hepatic damage, and hematuria have been reported. Eye and skin irritation are also possible. Tachypnoea occurs in glyphosate poisoning in other animals but does not appear to be a feature of glyphosate toxicity in dogs.

Studies to examine the effects of chronic feeding of glyphosate to Wistar rats have measured the activity of some enzymes with a function in the pathways of NADPH generation, isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase, and malate dehydrogenase in liver, heart, and brain of pregnant Wistar rats and their fetuses that were exposed to glyphosate solutions of 0.5% and 1% at a dose of 0.2 and 0.4 mL/mL water during 21 d of pregnancy. Glyphosate affects these enzymes in the studied organs of pregnant rats and their fetuses (Daruich et al. 2001).

Feeding Glyphosate-Biocarbo formulation at rates of 4.87 mg/kg every 2 d for 75 d resulted in leakage of the hepatic intracellular enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST), suggesting irreversible damage in hepatocytes (Benedetti et al. 2004). The formulation used in this study was from Brazil, and the identity and composition of the formulants are unknown. In addition, the exposures extended over a long period and were judged inappropriate for assessing risks from acute and infrequent exposures such as may occur in eradication spraying.

The effect of glyphosate on the activity of several enzymes was studied *in vitro*. The enzymes measured were serum acetylcholinesterase (AChE), lactate dehydrogenase (LDH), aspartate amino-transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), and acid phosphatase (AcP). Glyphosate inhibited all enzymes except AcP. IC_{50} values were 714.3, 750, 54.2, 270.8, and 71.4 mM for ACHE, LDH, AST, ALT, and AP, respectively (El-Demerdash et al. 2001). The most sensitive response, that of AST, was observed at 54.2 mM, equivalent to a concentration of 9,056 mg/L, a concentration that would not occur *in vivo*. These results do not suggest that glyphosate would have effects at concentrations lower than those previously observed.

Glyphosate has not been found to be mutagenic, genotoxic, or carcinogenic. Glyphosate was not teratogenic or developmentally toxic except at large exposures (Williams et al. 2000). Some studies that were not reviewed by Williams et al. or were published after 2000 are reviewed below.

In a study on Charles River CD-1 rats, test animals were given oral gavage doses of 0, 300, 1,000 and 3,500 mg/kg bw/d of glyphosate from day 6–19 of gestation. Control animals received 0.5% methocel. No internal or skeletal anomalies were seen at 300 and 1,000 mg/kg bw/d, although maternal toxicity was apparent at 3,500 mg/kg bw/d with soft stools, diarrhea, red nasal discharge, reduced body weight, and death by gestation day 17 (6/25). In addition, mean fetal body weights were significantly reduced and early fetal resorption was significantly increased at this dose (Rodwell 1980b). Female Dutch belted rabbits were given oral gavage doses of 0, 75, 175, and 350 mg/kg bw/d glyphosate from day 6–27 of gestation. Control animals received 0.5% methocel. No internal or skeletal abnormalities were seen (Rodwell 1980a). In a study from Brazil, examination of pregnant Wistar rats dosed orally with Roundup from day 6–15 of pregnancy with rates of 0, 500, 750, or 1,000 mg/kg bw glyphosate showed skeletal alteration in fetuses (15.4%, 33.1%, 42.0%, and 57.3%, respectively). There was 50% mortality of dams at 1,000 mg/kg only (Dallegrave et al. 2003). The doses were large and considerably greater than those used in an earlier study (reviewed by Williams et al. 2000). In the earlier study, a no-observed-effect-level (NOEL) of 15 mg/kg bw/d was described for fetal effects and 300 mg/kg bw/d for maternal effects. Given the very large doses used in the Dallegrave et al. (2003) and Rodwell studies (1980), their results are not surprising and do not change the assessment of teratogenic potential in Williams et al. (2000).

A number of recent studies have been carried out in tissue culture. One assessed the affect of several formulated pesticides on the steroidogenesis pathway (StAR protein synthesis) in tissue cultures of mouse testicular Leydig tumor cells (Walsh et al. 2000). Exposure to the formulation at 25 mg/L in the cell culture medium caused a reduction in steroidogenesis but only for a period less than 24 hr during which there was recovery. In another study, Lin and Garry reported results of bioassays carried out in cultures of the MCF-7 breast cancer cell (Lin and Garry 2000). Results indicated that although some pesticides caused estrogen-like receptor-mediated effects at large exposure concentrations, both glyphosate and the Roundup formulation induced nonestrogen-like proliferation, thereby supporting the view expressed by others (Williams et al. 2000) that neither glyphosate nor Roundup is an endocrine disruptor.

Studies on cells *in vitro* are difficult to interpret as they exclude the normal pharmacokinetic and metabolic functions that would be present in whole animals; thus, these should be compared to the multigenerational study used by regulatory agencies worldwide to assess reproductive/developmental toxicity, which is the most definitive study design for the evaluation of potential endocrine modulating substances in humans and other mammals. Comprehensive reproductive and developmental toxicology studies carried out in accordance with internationally accepted protocols have demonstrated that glyphosate is not a developmental or reproductive toxicant and is not an endocrine disruptor (Williams et al. 2000; USEPA 1993a; World Health Organization International Program on Chemical Safety 1994).

There was no evidence of neurotoxicity in a number of studies reviewed in Williams et al. (2000). Neurotoxicity was not observed in the large number of acute, subchronic, and chronic studies conducted in rodents nor was it observed in two specific neurotoxicity studies conducted in dogs. However, these studies did not assess potential effects on neurotransmitters and their metabolites in the brain and other parts of the nervous system, measures of response used in current testing protocols for neurotoxicity.

Some reports on the immunotoxicity of glyphosate appear in the literature. Female CD-1 mice exposed to Roundup at concentrations up to 1.05% in drinking water for 21 d showed no change in immune function (T-lymphocyte and macrophage-dependent antibody response) when, on day 21 of the exposure period, they were inoculated with sheep erythrocytes (Blakley 1997). In an *in vitro study* on cytokine production by human peripheral blood mononuclear cells, glyphosate had only a slight effect at the greatest concentration tested (1,000 µM = 226,000 µg/L) (Nakashima et al. 2002). Results of both studies suggest that glyphosate does not affect immune response in mammals at realistic exposure concentrations. However, studies in fish suggest that that there may be some immunotoxic effects. Short exposures to Roundup (10 min at a concentration of 100,000 µg/L) in carp (*Cyprinus carpio*) and European catfish (*Silurus glanis*) caused a decrease in metabolic and phagocytic activity as well as proliferative response (Terech-Majewska et al. 2004). In contrast to these effects at large concentrations, responses on splenic antibody plaque-forming cells in the fish *Tilapia nilotica* were reported at concentrations of 1.65×10^{-2} µM (= 4.4 µg/L). As responses of the immune system are difficult to interpret in terms of survival of individuals or the population, they are not formally used in assessment of pesticides by regulatory agencies.

Toxicokinetics of glyphosate were reviewed by Williams et al. (2000). Between 15% and 36% of ingested glyphosate is absorbed through the intestinal tract and only about 2% via the skin. Excretion of unabsorbed glyphosate is via the feces, but the absorbed glyphosate is excreted via the urine with only a small amount of metabolism. Whole-body half-lives were biphasic, with an initial half-life of 6 hr and a terminal elimination half-life of 79–337 hr in rats (Williams et al. 2000). Clearance from most tissues was rapid but was cleared more slowly from the bone, possibly because of ionic binding to bone calcium (Williams et al. 2000). Glyphosate is clearly not bioaccumulated, and any absorbed dose is excreted in the urine relatively rapidly.

Cases of Human Poisoning. A number of anecdotal reports of human poisoning with glyphosate and its formulations have been published. In some cases, these are reports of a single event and an observed response.

In one, toxic pneumonitis was observed after exposure to a glyphosate formulation (Pushnoy et al. 1998). However, no information was provided to demonstrate how airborne exposure could have occurred and the results are at odds with the known inhalation toxicity of the formulation (Williams et al. 2000) and tests done on the product as used in Colombia (see following).

In another case, a man accidentally sprayed himself with an unidentified formulation of glyphosate (Barbosa et al. 2001). He developed skin lesions 6 hr after the accident but these responded to routine treatment. However, 1 mon later, the patient presented with a case of symmetrical Parkinsonism syndrome. This is an isolated case, and it is impossible to conclude anything about causality as the disease may have already been present but asymptomatic. In a similar case, a 78-yr-old woman presented with extensive chemical burns in legs and trunk caused by an accidental contact with a glyphosate formulation. These lesions disappeared, without consequences, a month later (Amerio et al. 2004).

Acute intoxication information has been documented in two case-series studies, from Taiwan, China, where glyphosate formulations were apparently used for attempted suicide (Chang et al. 1999; Lee et al. 2000). The first paper analyzed 15 intentional intoxications with glyphosate formulation and found that 68% of the patients presented esophageal, 72% gastric, and 16% duodenal injuries. Esophageal injury was the most serious injury but was minor in comparison with that caused by strong acids. Lee et al. (2000) analyzed 131 suicide attempts in southern Taiwan. The most common symptoms were sore throat and nausea; the fatality rate was 8.4%. In this study, 20.5% presented respiratory symptoms and more than half of them needed intubations. The authors propose that direct damage to the airway passage occurs and mention that the surfactant in the formulation (POEA MON 0818) may be responsible for the toxicity. In many cases, the exact doses consumed by persons attempting suicide are not known, and it is difficult to interpret these findings in the context of bystander and other accidental exposures, which are usually many orders of magnitude less. It is, however, interesting to note the low fatality rate compared to what has been reported from other pesticides such as paraquat and the organophosphorus insecticides (Krieger 2001).

It is well known that the older formulations of glyphosate that contained the surfactant POEA (MON 0818) in larger amounts were eye irritants. Goldstein et al. (2002) analyzed 815 glyphosate-related "calls" to the Pesticide Illness Surveillance Program (PISP), most of them involving eye irritation (399), skin (250), upper airway (7), and combinations of these. Of the 187 systemic cases, 22 (12%) had symptoms definitely related to exposure to formulations of glyphosate. Again, this is not surprising as the formulation of glyphosate is acidic, similar to strong vinegar, and the surfactant is an eye irritant. In other studies on eye and skin irritation reviewed in Williams et al. (2000), none of the reported exposures resulted in permanent change to the structure or function of the eye. Based on these findings, it was concluded that the potential for severe ocular effects in users of Roundup herbicides is extremely small. This observation is consistent with the minimal ocular and dermal effects observed with the formulation of glyphosate used in Colombia (see below).

Human Epidemiology Studies. A number of studies in the recent epidemiology literature have attempted to address the issue of glyphosate exposure and disease incidence in humans. Epidemiology studies on pesticides commonly suffer from two sources of error. Possibly the most important of these is the error in assigning exposures. Exposures in the studied population are never measured directly and it is common to use surrogates for exposures such as areas treated with pesticides, number of applications, and/or number of years of application. Studies have shown that these surrogates are susceptible to significant errors (Arbuckle et al. 2004). Similar conclusions have been put forward by others (Arbuckle et al. 2005; Harris et al. 2002; Solomon et al. 2005b). A second possible source of error is the fact that the populations that are studied (farmers and professional applicators) typically use many pesticides. Thus, any substance-specific responses and causality are difficult to ascertain.

Cancer Studies. The work of Hardell et al. (2002) presented a pooled analysis of two case-control studies, one on non-Hodgkin's lymphoma (NHL) (Hardell and Eriksson 1999) and another related to a hairy cell leukemia (HCL), a rare subtype of NHL. The 1999 study employed a case-control study design based on a total of 442 subjects; however, only 4 cases and 3 controls, or less than 1% of the overall study subjects, reported the use of glyphosate. The conclusions are thus based on small numbers and the confidence interval (CI) reported for exposure to glyphosate was 0.4–13, showing a lack of statistical confidence. In their pooled analysis, Hardell et al. (2002) reported a positive association with use of glyphosate [odds ratio (OR) 3.04, 95% CI of 1.08–8.52] when analyzed using univariate statistics with the highest risk for exposure during the latest decade before diagnosis. However, the OR was reduced when using multivariate statistics (OR 1.85, 95% CI of 0.55–6.20). In addition, the study was based on a small number of cases and controls (8/8) and lacked power to differentiate linkages.

De Roos et al. (2005) evaluated associations between glyphosate exposure and cancer incidence in the Agricultural Health Study (AHS), a prospective cohort study of 57,311 licensed pesticide applicators in Iowa and North Carolina. Among private and commercial applicators, 75.5% reported having ever used glyphosate, of which >97% were men. In their analysis, glyphosate exposure was defined as (a) ever personally mixed or applied products containing glyphosate, (b) cumulative lifetime days of use, and (c) intensity-weighted cumulative exposure. Glyphosate exposure was not associated with incidence of 12 common cancer types [the relative risk (RR) included 1 in all cases]; however, the RR for multiple myeloma incidence was 2.6 (95% CI of 0.7–9.4 based on 32 cases of the total of 2,088 cancers), prompting the authors to suggest that this should be followed up in future studies.

Overall, there is no strong evidence to link glyphosate exposure to increased risk of cancer. Taken with the lack of any evidence of genotoxicity or carcinogenicity of glyphosate in laboratory studies (Williams et al. 2000), it is highly unlikely that glyphosate is carcinogenic in humans.

Neurological Effects. A recent study on farmers in the Red River Valley in Minnesota (USA), reported on the link between glyphosate and attention deficit disorder and attention deficit hyperactivity disorder (ADD/ADHD) in children of farmers who applied it (Garry et al. 2002). They reported an OR of 3.6 (95% CI, 1.3–9.6); however, the study suffered from several potential sources of error. The authors noted the lack of uniform diagnostic neurobehavioral information related to ADD/ADHD and that their study identified 14 cases of ADD/ADHD among 1,532 live births, a frequency that was actually considerably lower than background rates of ADD/ADHD which had previously been reported by researchers in Canada and the U.S. Notwithstanding, while Garry et al. (2002) concluded that their study showed a tentative association between ADD/ADHD and the use of glyphosate, they also noted that other experimental evidence did not support this conclusion, including that glyphosate was not genotoxic and that little, if any, evidence of neurotoxicity has been associated with exposure to glyphosate, except in cases of intentional oral overdose. Finally, the authors expressed concern that their tentative conclusions could be explained by random chance alone and stated the need for further detailed neurodevelopmental studies to resolve these outstanding issues. Overall, there appears to be little evidence to support a link between glyphosate exposure and neurobehavioral problems in children of exposed applicators.

Reproductive Outcomes. Several papers have reported on the relationship between adverse reproductive outcomes and the use of glyphosate. In a study in Ontario, Canada, Arbuckle et al. (2001) observed a moderate increase in the risk of late abortions associated with preconception exposure to glyphosate (OR = 1.7, 95% CI, 1.0–2.9). Another study in Ontario, part of the Ontario Farm Family Health Study, reported a positive association (decrease in fecundability of 20%, ratio range = 0.51–0.80) when both spouses participated in activities where they could be exposed to pesticides. This finding was observed for 6 of 13 pesticide categories, 1 of which was glyphosate (Curtis et al. 1999). The study was based on 2,012 planned pregnancies. There was no strong or consistent pattern of associations of pesticide exposure with time to pregnancy (TTP). For exposure intervals in which only the men participated in pesticide activities or in which neither men nor women participated in pesticide activities but pesticides had been used on the farm, conditional fecundability ratios ranged from 0.75 to 1.50, with no apparent consistency among pesticide classes, chemical families, or active ingredients. Again, although this study did suggest a linkage between pesticide exposure and fecundability, there is no evidence from laboratory studies that glyphosate is a reproductive toxicant at exposures that would be expected in humans (Williams et al. 2000).

Overall, there is little epidemiological evidence to link glyphosate to any specific diseases in humans. This conclusion is supported by laboratory toxicity studies. However, responses related to reproductive outcomes such as fecundability measured through time to pregnancy offer a useful measure of possible effects that can be applied in situations such as Colombia where other health data are difficult to gather. With this in mind, a preliminary study was designed to gather human epidemiological data in several regions in Colombia. These regions were the same as those selected for the surface-water sampling (see Table 9). The design and results are summarized in the following section; a detailed report is given in a separate document (Sanin 2005).

Human Health Effects – Time to Pregnancy. A specific study was conducted to elucidate possible effects on reproductive health from exposure to glyphosate and adjuvants by assessing fertility/fecundability among women resident in different areas of the country with different pesticide use patterns. The design was cross-sectional with retrospective collection of data and is equivalent to a retrospective cohort. The study population consisted of 600 women of reproductive age in each of five different areas (see Table 9). The independent variable in the study was exposure to glyphosate for control of illicit crops, measured through use information from the region as indicated in Table 9. Possible confounders or independent predictors of reproductive variables in study the were also considered (Sanin 2005; Solomon et al. 2005a).

The distribution of pregnancies in relation to time to pregnancy (TTP) (Fig. 8) was different among the five regions. In previous work in Colombia (Idrovo et al. 2005), the percentage of pregnancies for first month was about 30%, small compared with data from developed countries. In this case, Valle del Cauca had very small initial percentage and Boyacá had larger values for the 1st and 12th months (Fig. 8). The mean for 12 mon in developed countries is 85%–90%.

In the crude analyses, longer TTP was associated with a number of factors such as region, older maternal age, ethnic group, irregular menstrual cycles, and irregular partner relationship. Previous visits to a physician for problems related with fertility, X-rays taken in the year before pregnancy (YBP), and coffee consumption in the YBP also were associated with longer TTP. Coffee consumption had a significant test for trend, but the odds ratio (OR) was not significant. Maternal overweight was associated with a longer TTP. A tendency to longer TTP was observed among those

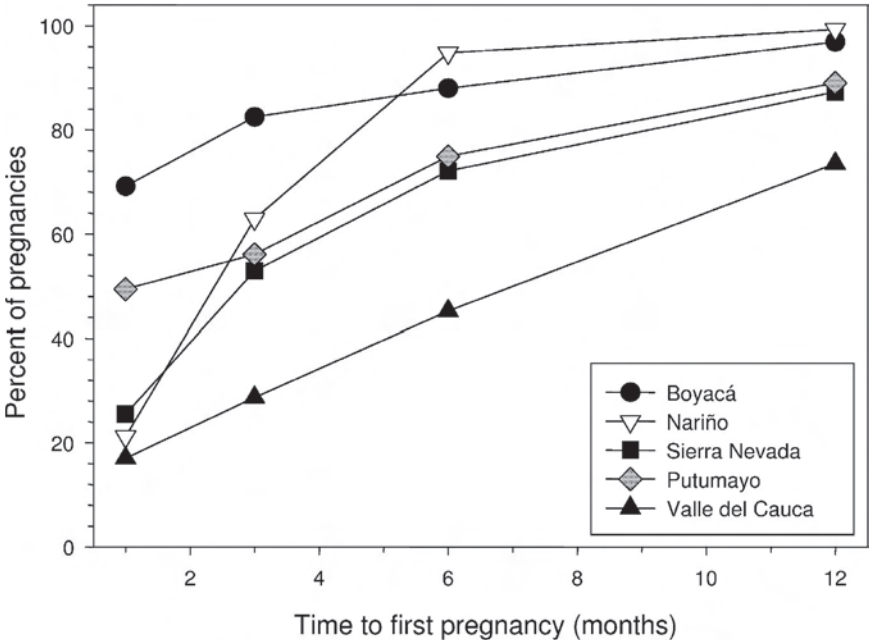


Fig. 8. Time to pregnancy (TTP) in the five study regions in Colombia.

engaged in some waged work and with higher education. Paternal unemployment or self-employment was associated with longer TTP. No other paternal data were related with the TTP.

In the final multivariate model, the main predictor of TTP was the region adjusted by irregular relationship with partner and maternal age at first pregnancy. Boyacá had the minimal risk and was the reference region; Nariño, Sierra Nevada, and Putumayo had slightly greater risk. The greatest risk was in the Valle del Cauca region. There was no association between TTP and use of herbicides in the control of illicit crops in the regions studied. The reason(s) for the increased risk for longer TTP in the Valle del Cauca region, where sugar cane is grown, is not known. In this study, the increased risk in Valle del Cauca cannot be attributed to exposure to pesticides alone because Sierra Nevada, where organic crops are grown, also showed a statistically significant difference from the reference location where pesticides are used (Boyacá). This study was designed to test hypotheses related to the use of glyphosate in eradication spraying, and the data cannot be used to identify causality associated with other risk factors. To test this question in Valle del Cauca or any other region, a new study would have to be designed and conducted. Some of the factors associated with higher TTP that were identified should be included in any future studies.

Effects of Glyphosate in Nontarget Organisms in the Environment

The mechanism of action of glyphosate is via the disruption of the shikimate metabolic pathway that leads to the synthesis of aromatic compounds in numerous microorganisms and plants. Glyphosate translocates to active growing tissues; this is particularly effective in most plants because its degradation is slow. Thus, the herbicide moves throughout the plant before symptoms are noticed. The shikimate pathway is absent from mammals (Eschenburg et al. 2003; Roberts et al. 1998, 2002). However, toxic effects of the compound on, for example, nonmammalian aquatic organisms, have been observed at large concentrations. These effects are discussed in more detail below.

A common question in conducting risk assessments in tropical regions and other nontemperate regions is the paucity of toxicity data for "tropical species." It is true that most of the test species used in toxicity testing, particularly of pesticides, are "temperate species" largely because of the location of testing laboratories that are able to conduct guideline toxicity tests under Good Laboratory Practice (GLP). Except for a few substances with defined mechanisms of action, there is no reason to believe that organisms from tropical regions are inherently more or less sensitive than organisms from temperate regions. It is well known that DDT and some of the pyrethroids become more toxic at lower temperatures (Dyer et al. 1997); however, the mechanisms here are well understood and do not apply to glyphosate. Comparison of responses of tropical and temperate organisms to a number of pesticides other than DDT has shown that there are no significant differences in sensitivity (Maltby et al. 2005). With this in mind, we used the rich data set of toxicity values that have accumulated in the literature for glyphosate and its formulations.

Effects in Nontarget Terrestrial Animals. The potential environmental effects of glyphosate and Roundup were extensively reviewed in 1999 (Giesy et al. 2000). Additional papers have appeared since then.

Soil Invertebrates. The effects of glyphosate and formulations on earthworms have been reviewed (Giesy et al. 2000) and risks were judged to be essentially negligible. A recent study on the earthworm *Eisenia fetida* reported that, although a commercial formulation of glyphosate was not directly toxic to the earthworms, it did cause effects on locomotory activity that may be detrimental to the earthworms (Verrell and Van Buskirk 2004). The formulation used in the study was Ortho Groundclear Total Vegetation Killer, which contains 5% by volume glyphosate as the isopropylamine salt (IPA). In this study, the authors applied 82 mL of a 1 : 4 solution of Groundclear to 2 L of soil in a plastic box, an amount much greater than would be applied under normal agricultural uses or in the control of illicit crops. Assuming that the boxes of soil were cubes, the area of the surface would be 12.6 × 12.6 cm or 159 cm^2. This being so, the application rate was equivalent to 518 kg glyphosate/ha, a totally unrealistic application rate and 100 times more than that used in the control of coca. This study was seriously flawed, and the results are not applicable to any field use of glyphosate.

Soil Microorganisms. Glyphosate and its formulations have little effect on soil microorganisms (Giesy et al. 2000). Because the symbiotic soil and root-associated microorganisms may be partially dependent on the plant for nutrients, the death or injury of the plant will affect the organisms associated with it. Similarly, death of the plants will release organic matter and nutrients into the soil, affecting soil microorganisms similarly to the application of compost or fertilizer. This response, as reported for glyphosate formulation and its effects on grass (Tenuta and Beuchamp 1995), would also occur with other herbicides and with mechanical control of plants. Effects have been demonstrated in hydroponically grown plants exposed through the watering solution; however, this route of exposure is not relevant to field conditions where glyphosate would bind strongly to soil particles and not be biologically available. Effects on symbiotic microbiota have also been demonstrated in glyphosate-tolerant plants treated at 10 times normal field application rates, but these are not relevant exposures as the studies were done in vitro and in the absence of soil (Mårtensson 1992). Some effects on metabolism of phenolic substances in symbiotic bacteria in glyphosate-tolerant soybeans have been shown; however, these changes did not alter nitrogenase activity (Hernandez et al. 1999). Microbial systems in soil are complex, and considerable variation can be expected among tests and among soil types. More-recent studies on the effects of glyphosate on microbiological activity in soils have shown an increase in microbiological activity, mainly in fungi, which are likely using the glyphosate as a source of carbon, nitrogen, and phosphorus (Araujo et al. 2003; Haney et al. 2002; Laatikainen and Heinonen-Tanski 2002). These changes in microbiological activity are not judged to be deleterious.

The effects of several fungicides and herbicides on the growth of the ectomycorrhizal fungi *Lactarius deliciosus*, strain LDF5, and *Pisolithus tinctorius*, strains 30AM, 3SR, and Mx, in pure culture have been studied. Glyphosate (formulation unspecified) at concentrations of 0, 1, 10, 100, and 1,000 mg/kg soil had no effect (Diaz et al. 2003). Some 64 strains of ectomycorhizal fungi were tested against the most common pesticides used in forestry in Finland. Glyphosate did not produce strong inhibition in any of the strains, most were unaffected, and some were stimulated by 1 mg/L Roundup Bio in agar (Laatikainen and Heinonen-Tanski 2002). Laboratory tests on four species of entomopathogenic fungi have shown that glyphosate has no effect, but a range of formulated products did have fungicidal properties, especially RoundUp Ready-To-Use (Morjan and Pedigo 2002). In fact, as fungi and bacteria have the shikimate pathway, this suggests the potential use of shikimate pathway inhibitors for the beneficial control of fungal pathogens and apicomplexan parasites, such as *Toxoplasma gondii*, *Plasmodium falciparum*, and *Cryptosporidium parvum* (Roberts et al. 1998, 2002).

Analysis of all lines of evidence for effects of glyphosate and its formulations on soil microorganisms indicates that adverse effects would be unlikely as a result of application at normal field rates. Any minor effects to communities, such as described above, would be expected to disappear rapidly (Giesy et al. 2000; World Health Organization International Program on Chemical Safety 1994). After reviewing several studies conducted in many climates, in different soils over the past 10 years, and under various cropping systems, Motavalli et al. (2004) have concluded that, so far, glyphosate and its formulations have no relevant effect on nutrient transformations by microbes. However, they point out that this topic needs further study, as not every situation has been adequately researched. Further, because of lack of bioavailability on soils, adverse effects on beneficial soil fungi and bacteria are unlikely to occur under field conditions. Glyphosate binds strongly to soil particles and would not be available for uptake by microorganisms, many of which are actually inside the plant tissues. The fact that seeds will readily germinate in soils soon after treatment with glyphosate and that nitrogen-fixing Roundup Ready soybeans grow and develop high yields despite treatment with glyphosate demonstrates the practical insignificance of these effects under actual use conditions.

Terrestrial Invertebrates. As glyphosate is a nonselective herbicide, it will cause habitat alteration, which also results from a number of human activities in the production of food and fiber. Most important is the clearing of land for agricultural production. Whether this is through slash-and-burn processes as used in the initial preparation of coca and poppy fields or the application of herbicides such as glyphosate and paraquat, also used in coca production, the effects on nontarget species are the same. Use of cultural or mechanical controls, or herbicides, to remove plants will have effects on organisms that normally use these plants for food or shelter.

After applying glyphosate formulation at double the recommended application rates, no effects were observed in microarthropods in soil (Gomez and Sagardoy 1985). Because weed species compositions and densities are directly affected by glyphosate, indirect effects are more likely to occur. Jackson and Pitre (2004b) found that populations of adult *Cerotoma trifurcata*, adult *Spissistilus festinus*, larvae of *Plathypena scabra*, and the caterpillar of *Anticarsia gemmatalis* were unaffected by glyphosate formulation, but populations of adult *Geocoris punctipes*, a homopoteran insect predator, were decreased. This effect was caused by reduced weed densities after herbicide treatment. Populations of green cloverworm (*Hypena scabra*) were evaluated on soybean glyphosate-resistant varieties, with and without exposure to glyphosate [glyphosate acid Roundup Ultra at 2.48 kg equivalent per hectare (AE/ha)], and no differences among treatments were detected on developmental time and survivorship (Morjan and Pedigo 2002). Weed management systems, more than glyphosate, that allowed more weeds to grow generally had higher insect population densities (Buckelew et al. 2000).

Effects of glyphosate and associated cultural practices can affect arthopods indirectly. In studies conducted in the U.K., indirect effects of glyphosate were observed in the spider *Lepthyphantes tenuis*, caused by habitat alteration and related to death of plants and decreasing height of vegetation. Applications of glyphosate had only a within-season indirect habitat effect on *L. tenuis* as field margins sampled 16 mon after an application of 360 g glyphosate AE/ha showed no detrimental effects (Bell et al. 2002; Haughton et al. 2001). Tests of the fecundity and mortality of *Geocoris punctipes* (Say), exposed to glyphosate as Roundup on soybean found no effects over 10-d posttreatment. Exposure of *G. punctipes* eggs to Roundup Ultra spray had no effect on egg hatch (Jackson and Pitre 2004a). Some reductions in numbers of this species 3 wk after treatment probably reflect weed removal (Jackson and Pitre 2004b).

Similarly, studies on populations of leaf litter invertebrates in areas of Australia where Roundup Biactive was sprayed at 1–1.4 kg/ha for the control of an invasive weed showed no significant effects 4 mon after spraying (Lindsay and French 2004). Variability in treated and untreated areas was large and suggested that the nature of the vegetative community and its structure and the postspray weather may also be important. In agriculture, these effects are part of the risk assessment related to integrated pest management (IPM), and potential effects on beneficial organisms are weighed in the risk–benefit equation. In conclusion, there is little evidence of any direct effect of glyphosate on insects in the field or in natural environments.

Terrestrial Vertebrates. Technical glyphosate, formulated glyphosate (above), and glyphosate mixed with Cosmo-Flux (see below) are not acutely toxic to mammals via several routes of exposures. Although wild mammals have not been specifically tested with the mixture as used in Colombia, data from these laboratory studies suggest that they would be insensitive and not directly affected by a direct overspray.

Birds are not susceptible to glyphosate. In studies on bobwhite quail (*Colinus virginianus*) and mallard duck (*Anas platyrhynchos*), acute oral LD_{50} values of >4,640 and >4,640 mg/kg bw have been reported (USEPA 2001). Again, direct effects of formulated glyphosate or glyphosate plus Cosmo-Flux are judged to be very unlikely.

Indirect effects on terrestrial wildlife have been reported with the use of glyphosate in agriculture and forestry. Alteration of habitat is more of an issue in semiwild areas such as forests where herbicides may be used to control competing vegetation and allow conifers to grow and mature more rapidly. In these cases, short-term effects on birds and other wildlife do occur; however, these populations usually recover in 2–3 yr (Kimball and Hunter 1990; Santillo et al. 1989a,b), and even the vegetation recovers in less than 10 yr (BC Ministry of Forests 2000; Boateng et al. 2000). Normally, in these uses, the actual areas treated are relatively small and are surrounded by or adjacent to untreated areas that can act as refugia or sites for repopulation by animals that have moved away because of the changes in habitat. As new vegetation develops to replace that controlled by the herbicide, the habitat will again become usable by these animals (Giesy et al. 2000; World Health Organization International Program on Chemical Safety 1994).

Glyphosate is widely used for vegetation management, including the restoration of native plant communities where exotic or invasive species are controlled (Hartman and McCarthy 2004). The use of glyphosate for "conifer release" from competition has minimal effects on wildlife and can be used to enhance biodiversity if used for spot and patch treatments (Sullivan and Sullivan 2003). A review of management of northern U.S. forests, including the use glyphosate, indicated no adverse ecological effects (Lautenschlager and Sullivan 2002). However, the impacts of vegetation removal by manual clearance and glyphosate application in conifer plantations had effects on bird communities in British Columbia, mediated by the removal of deciduous plants. Where the herbicide was used, number of bird species declined, total number of individuals increased, and common species dominated. Populations of residents, short-distance migrants, ground gleaners, and conifer nesters increased significantly after herbicide treatment. Deciduous nesters and foliage gleaners increased in abundance (nonsignificantly) in control and manually thinned areas. Warbling vireos (*Vireo gilvus*), which are deciduous specialists, declined in treated areas and may be particularly susceptible to the indirect effects of glyphosate plant removal (Easton and Martin 1998; 2002).

Nevertheless, control of *Cirsium arvense* (Canada thistle) using wick application of glyphosate in wildfowl areas can enhance plant diversity that benefits water birds (Krueger-Mangold et al. 2002). However, the broad-spectrum activity of glyphosate means that accidental overspray of rare nontarget plant species during control of invasive plants will cause damage (Matarczyk et al. 2002).

Beneficial Insects. Glyphosate is not considered toxic to honeybees, with a reported LD_{50} of >100 µg/bee (USEPA 2001), however, the formulation, with the adjuvant Cosmo-Flux, as used in Colombia may have different toxicity because of the added surfactants. To test this hypothesis, toxicity testing of a mixture of a commercial formulation of glyphosate and the surfactant Cosmo-Flux 411F was conducted to determine the acute contact toxicity to honey bees (*Apis mellifera* L.) (Stantec 2005a), following standard test guidelines (OECD 1998a; USEPA 1996a). The mixture of glyphosate and Cosmo-Flux 411F was not toxic via acute contact exposure to honeybees (i.e., did not cause mortality or stress effects in bees within 48-hr of treatment) at concentrations equal to or less than 63.9 µg AE/bee. These results are similar to those for glyphosate and formulations from the USEPA ECOTOX data base (USEPA 2001) and show that the formulated product as used in Colombia is not hazardous to bees or, by extrapolation, to other beneficial insects.

Effects in Aquatic Animals. Several extensive reviews of the effects of glyphosate on aquatic organisms have concluded that glyphosate presents an essentially negligible risk to aquatic organisms (Giesy et al. 2000; Solomon and Thompson 2003; World Health Organization International Program on Chemical Safety 1994). Several publications reported on the effects of glyphosate and several of its formulations in frogs. The acute toxicity of technical-grade glyphosate acid, glyphosate isopropylamine, and three glyphosate formulations to Australian frog species was measured by Mann and Bidwell (1999). Acute toxicity was observed for adults of one species and tadpoles of four species of southwestern Australian frogs in 48-hr static/renewal tests. The 48-hr LC_{50} values for Roundup herbicide (MON 2139) tested against tadpoles of *Crinia insignifera*, *Heleioporus eyrei, Limnodynastes dorsalis*, and *Litoria moorei* ranged between 8,100 and 32,200 µg/L (2,900 and 11,600 µg AE/L, whereas the 48-hr LC_{50} for Roundup herbicide tested against adult and newly metamorphosed *C. insignifera* ranged from 137,000 to 144,000 µg/L (49,400–51,800 µg AE/L). These values were different, depending on the type of dilution water (lake or tap water). For the purposes of this risk assessment, the most sensitive stage was used.

Touchdown herbicide (4 LC-E) tested against tadpoles of *C. insignifera*, *H. eyrei*, *L. dorsalis*, and *L. moorei* was slightly less toxic than Roundup with 48-hr LC_{50} values between 27,300 and 48,700 µg/L (9,000 and 16,100 µg AE/L, respectively). Roundup Biactive (MON 77920) was practically nontoxic to tadpoles of the same four species, producing 48-hr LC_{50} of 911,000 µg/L (328,000 µg AE/L) for *L. moorei* and >1,000,000 µg/L (>360,000 µg AE/L) for *C. insignifera*, *H. eyrei*, and *L. dorsalis*. Technical glyphosate isopropylamine salt was practically nontoxic, producing no mortality among tadpoles of any of the four species over 48-hr, at concentrations between 503,000 and 684,000 µg/L (343,000 and 466,000 µg AE/L). The toxicity of technical-grade glyphosate acid (48-hr LC_{50}, 81,200–121,000 µg AE/L) is likely to be caused by acid intolerance. Slight differences in species sensitivity were evident, with *L. moorei* tadpoles showing greater sensitivity than those of the other four species. Adult and newly emergent metamorphs were less sensitive than tadpoles.

A series of studies on frogs was conducted with several formulations of glyphosate in relation to its use in forestry in Canada (Chen et al. 2004; Edginton et al. 2004; Thompson et al. 2004; Wojtaszek et al. 2004). Using a formulation of glyphosate (Vision) containing glyphosate and ethoxylated tallowamine surfactant (POEA), LC_{50} values as low as 880 µg AE/L were reported for tadpoles of *Xenopus laevis*, *Bufo americanus*, *Rana clamitans*, and *Rana pipiens* (Edginton et al. 2004). Embryo stages were less sensitive than older larvae, and toxicity was affected by the pH of the exposure medium, although not in a consistent manner. For the purposes of this assessment, values obtained at the most sensitive pH and for the most sensitive stage were used.

In a related study on the toxicity of the Vision formulation of glyphosate to the zooplankton organism *Simocephalus vetulus* and tadpoles (Gosner stage 25) of *Rana pipiens,* interactions between pH and food availability were reported (Chen et al. 2004). Both high pH (7.5 vs. 6.5) and food deprivation increased the toxicity of this formulation. As only two concentrations were tested (750 and 1,500 µg AE/L), LC_{50} values could not be determined.

Field studies conducted on larvae of *R. clamitans* and *R. pipiens* with Vision showed that, in the presence of natural factors such as sediment and environmentally relevant pH, toxicity of the formulation was reduced compared with laboratory observations (Wojtaszek et al. 2004). The authors reported 96-hr LC_{50} values ranging from 2,700 to 11,500 µg AE/L (Wojtaszek et al. 2004). Although they used a formulation of glyphosate containing the more-toxic surfactant POEA, the results confirm that, in the presence of sediments, reduction in the bioavailability of glyphosate (and formulants) occurs, further reducing risks, a conclusion reached for this forestry use (Thompson et al. 2004) but which is equally relevant to the use of glyphosate in Colombia. These observations are consistent with the rapid dissipation of both glyphosate and the POEA surfactant in the presence of sediments (Tsui and Chu 2004; Wang et al. 2005).

Toxicity of a number of glyphosate formulations to frogs (*R. clamitans, R. pipiens, Rana sylvatica*, and *Bufo americanus*) was reported (Howe et al. 2004); these included Roundup Original, glyphosate technical, the POEA surfactant used in some glyphosate-based herbicides, and five newer formulations of glyphosate. As expected, the most toxic of the materials was the POEA surfactant, followed by Roundup Original, Roundup Transorb, and Glyfos AU. No significant acute toxicity was observed with glyphosate technical material [96-hr $LC_{50} > 17{,}900$ µg/L(AE)]. LC_{50} values for Roundup Original in *R. clamitans*, *R. pipiens*, and *R. sylvatica* were 2,200, 2,900, and 5,100 µg AE/L, respectively; these values were used in this risk assessment. Several other formulations of glyphosate were also tested in *R. clamitans* (Roundup Biactive, Touchdown, and Glyfos BIO) and were essentially nontoxic with LC_{50} values >57,000 µg AE/L.

In a study on *Rana cascadae,* a 48-hr LC_{50} for Roundup (52% IPA) of 3,200 µg AI/L (2,336 µg AE/L) was reported using static exposures in glass tanks (Cauble and Wagner 2005). In a chronic exposure study with the same formulation over a 43-d period in glass tanks without sediment using a 7-d static renewal exposure with nominal concentrations of 730 and 1,460 µg AE/L, the authors found a number of effects such as decreasing time to death, increased mortality (8.6% and 51% at 730 and 1,460 µg AE/L, respectively), increased time to metamorphosis, and decreased weight of metamorphs. The relevance of these observations to exposures under field conditions must be considered. Under field conditions, glyphosate and its surfactants have been shown to bind strongly to sediments, which rapidly reduces concentrations, resulting in reduced toxicity. Thus, under use conditions in the field, exposures longer than the 48–96-hr used in acute tests are extremely unlikely. As no sediments were present in the glass tanks of this study, the chronic exposures used are not representative of what occurs in the field and are not applicable to the risk assessment for amphibians.

In a study of several commercial pesticide formulations in leopard frogs (*Rana pipiens*), green frogs (*R. clamitans*), bullfrogs (*Rana catesbeiana*), the American toad (*B. americanus*), and gray tree frogs (*Hyla versicolor*), effects of Roundup and interactions with other pesticides were reported (Relyea 2004). The Roundup used in this study contained the more-toxic POEA surfactant. Survival and growth over a 16-d period were not significantly affected by the glyphosate formulation at 1,000 μg AE/L but some species were affected at 2,000 μg AE/L. Some interactions were observed between glyphosate and other pesticides such as the insecticides diazinon, carbaryl, and malathion. One paper reported that a glyphosate formulation containing POEA was highly toxic to tadpoles of several species of frogs exposed under realistic conditions in small (1,000-L) field microcosms (Relyea 2005a). The tadpoles (wood frog, *Rana sylvatica*; leopard frog, *Rana pipiens*; American toad, *Bufo americanus*; gray tree frog, *Hyla versicolor*; and the spring peeper, *Pseudacris crucifer*) were exposed to 3,800 μg AE/L commercial glyphosate (unspecified) applied directly to the surface of the water. Application rate was equivalent to 16 kg AE/ha, an unrealistic value. At this concentration, glyphosate formulated with POEA would be expected to be lethal to tadpoles. The discussion in the paper that suggests that use of glyphosate may have adverse effects on frogs more generally is thus based on a flawed study design and is not supported by other data, as already discussed. In a laboratory study (Relyea 2005b) in which juvenile terrestrial stages of three different species (*R. sylvatica, B woodhousii folweri, and H. versicolor*) were exposed to direct applications of formulated glyphosate at 1.6 mg active ingredient (AI)/m^2 (1.2 mg AE/m^2) in plastic tubs, 79% mortality was observed after only 24-hr. The volume of formulation (6.9 mL) used to spray the tubs and the concentration of glyphosate (1.9%, IPA assumed) suggest that actual exposures were much greater than stated (91 mg AE/tub). Clearly, there were errors in the description of the methods, and the results of the study are uninterpretable.

Effects on other nontarget aquatic organisms have also been reported. In studies on the toxicity of glyphosate to several aquatic algae and zooplankton, Tsui and Chu (2003) showed that technical glyphosate was considerably less toxic than Roundup, which is formulated with the POEA surfactant. LC_{50} and EC_{50} values for technical glyphosate ranged from 5,890 to 415,000 μg AE/L. In tests conducted in the presence of sediment (Tsui and Chu 2004), they showed that biological availability of glyphosate was significantly reduced by binding to sediment. The reduction in concentration in pore water resulting from the sediments was proportional to the amount of organic carbon in the sediments.

Tests on the fish *Oreochromis niloticus* (Nile tilapia) exposed for 3 mon to sublethal concentrations (5,000 and 15,000 µg/L) of glyphosate as Roundup caused significant damage to gill, liver, and kidney tissue. The structural damage could be correlated to the significant increase ($P \leq 0.05$) in aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase activities in the second and third months of exposure. The results indicated that long-term exposure to Roundup at large, although at sublethal concentrations, had caused histopathological and biochemical alterations of the fish (Jiraungkoorskul et al. 2003). Because technical glyphosate was not tested, the contribution of surfactants to this response cannot be judged.

In studies on the freshwater mussel *Utterbackia imbecillis*, a commercial formulation of Roundup was reported to have little toxicity (24-hr LC_{50} of 18,300 µg/L and a NOEC of 10,040 µg/L–7,442 µg AE/L) to larval mussels (Conners and Black 2004). In studies on genotoxicity in these mussels, there was no significant difference in response between the control and mussel larvae treated at one-fourth the NOEC, ≈2,500 µg/L (1,850 µg AE/L).

Response of total free amino acid profiles of snails to glyphosate exposures has been studied (Tate et al. 2000), showing that exposure of the aquatic snails (*Pseudosuccinae columella*) to technical glyphosate at nominal concentrations of 1,000–10,000 µg/L led to increased egg laying and increased amino acid concentrations in tissues. Technical glyphosate was not particularly toxic with a 24-hr LC_{50} of 98,900 µg/L (72,200 µg AE/L). The effect on egg laying and amino acid concentrations was stimulative rather than adverse, but the authors speculate that it could lead to increases in incidence of diseases for which the snails are intermediate hosts. Increases in parasites may affect organisms in the environment. Similar stimulation was observed in the rotifer *Brachionus calyciflorus* in which growth rates and sexual and asexual reproduction were stimulated in the presence of glyphosate (formulation unknown) at ≥4,000 µg/L (growth) and ≥2,000 µg/L for reproduction and resting egg production (Xi and Feng 2004). Again, although stimulatory and not "adverse", the authors point out that increases in one species may affect other species indirectly.

In a study on grazing of the alga *Scenedesmus* spp. by the aquatic crustacean *Daphnia pulex*, technical glyphosate was shown to have no adverse effect, although it appeared to stimulate algae growth (Bengtsson et al. 2004). This stimulation was suggested to be caused by release of nitrogen and phosphorus from glyphosate metabolism by *Daphnia*. Similar stimulation was also seen in the effects of glyphosate (Rodeo, glyphosate IPA without surfactants) on the primary productivity of a natural phytoplankton algal assemblage dominated by species of diatoms and a dinoflagelate (Schaffer and Sebetich 2004). A 60% increase in productivity as measured by assimilation of $^{14}CO_2$ was observed at concentrations of 125, 1,250, and 12,500 µg/L, with no apparent concentration response. The authors speculate that the increase was caused by the release of nitrogen and phosphorus from the breakdown of glyphosate.

Effects of Glyphosate on Terrestrial Plants

There are differences in glyphosate uptake between different coca species and between young and mature plants of *Erythroxylum coca* and *E. novogranatense* (Ferreira and Reddy 2000). Absorption through the leaf is greater in young plants of both species and greater in *E. novogranatense*. Earlier studies showed that control of regrowth was better in *E. novogranatense* for equivalent rate of glyphosate (Ferreira et al. 1997). This study also indicated that defoliation of *E. coca* 24-hr before application resulted in no significant effect of glyphosate (applied up to 6.7 kg AI/ha) on regrowth. This result confirms that, as for other plants, uptake via the leaves is the major route of penetration into the plant.

A study on the control of the perennial weed pepperweed (*Lepidium latifolium*) has shown better control with glyphosate following mowing. The mechanism is via the better movement of glyphosate to roots from leaves lower in the canopy. Following mowing, the leaf distribution and the spray deposition are closer to the ground, giving better basipetal translocation to roots and better subsequent control (Renz and DiTomaso 2004). In forestry situations with an aerial application, spray deposition is typically much greater higher in the canopy (Thompson et al. 1997). Studies of glyphosate efficacy on annual weeds indicated that application during the day (0900 and 1800) gives best control (Martinson et al. 2002; Miller et al. 2003).

Resistance to glyphosate is known for an increasing number of species, including *Conyza canadensis* (Mueller et al. 2003), Illinois waterhemp (*Amaranthus rudis* and *A. tuberculatus*) (Patzoldt et al. 2002), *Eleusine indica* (Baerson et al. 2002), *Lolium multiflorum* (Perez and Kogan 2003), and *Lolium rigidum* (Neve et al. 2003a,b). Rates of evolution of resistance in the latter species are dependent on herbicide use patterns as part of crop production.

Nontarget impacts of glyphosate on seed germination and growth characteristics of the F_1 generation of treated wild plant species have been reported. Blackburn and Boutin (2003) noted effects on 7 of 11 species tested with 1%, 10%, or 100% of a 0.89 kg AI/ha label rate of glyphosate formulated as Roundup solution sprayed near seed maturity. Effects of glyphosate drift on rice seed germination were reported by Ellis et al. (2003), and May et al. (2003) noted reduced seed production in alfalfa in the year following applications 1.760 kg AI/ha for *Cirsium arvense* control. Nevertheless, applications at 0.420 kg AE/ha on susceptible soybean had adverse effects on sprayed plants but not on progeny (Norsworthy 2004). Subtle adverse effects of glyphosate on pollen viability and seed set in glyphosate-resistant cotton were noted by Pline et al. (2003). Pollen viability of glyphosate-resistant corn was also significantly reduced by glyphosate applied at 1.12 kg AI/ha, but yield and seed set were not significantly affected (Thomas et al. 2004). These data indicate that drift might cause subtle ecological changes to plant communities associated with changes in plant recruitment. However, this would be significant only for communities largely made up of monocarpic plant species (those that flower once and die, especially annuals) dependent on seeds for recruitment.

B. Glyphosate and Formulants

There are a number of formulations of glyphosate on the market that may contain a number of surfactants and other formulants (Giesy et al. 2000; Solomon and Thompson 2003; Williams et al. 2000). Normally, this would not be an issue in the risk assessment of a pesticide; however, in the case of glyphosate IPA, the active ingredient has little toxicity to nontarget organisms, thus making the surfactant toxicity more important for risk assessment. For example, tests on Ca^{2+}-activated ATPase and cholinesterase (ChE) activities in the nervous system of the slug *Phyllocaulis soleiformis* showed no effects of pure glyphosate IPA. An effect noted with the formulation Gliz 480CS was caused by nonglyphosate components of the formulation (da Silva et al. 2003). Technical-grade glyphosate at concentrations of 52 mM (870 mg/L) did not affect the protozoan *Tetrahymena thermophila* or the parasite *Ichthyophthirius multifiliis*. However, the commercial formulation Glyphosate was up to 100 times more toxic, reflecting data for fish species and other aquatic invertebrates and caused by surfactants in the formulation (Everett and Dickerson 2003).

Because the spray solution as used in the control of coca and poppy in Colombia contains surfactants as part of the glyphosate formulation as well as additional surfactants (Cosmo-Flux) added to the spray mix, the toxicity of the formulants and the adjuvants may interact to change the toxicity of the mixture. For this reason, standardized toxicity tests for mammals and environmental nontarget organisms were conducted with the spray mixture itself, as discussed next.

Effects of Formulated Glyphosate and Cosmo-Flux on Mammals

Two series of mammalian toxicity tests on the formulation of glyphosate and Cosmo-Flux were conducted. One set was conducted in the U.S. (Springborn 2003a–g) under good laboratory practices (GLP) and using quality control assurance as appropriate for regulatory decision making. The other studies (Immunopharmos 2002a–j) were conducted in Colombia, also in compliance with GLP and according to USEPA guidelines. These studies were reviewed in detail (Solomon et al. 2005a), and a number of conclusions were drawn for the mixture glyphosate and Cosmo-Flux sprayed on either poppy or coca.

- The acute oral and dermal LD_{50} was >5,000 mg/kg bw in the rat. Therefore, this formulation is considered as practically nontoxic orally.
- The acute inhalation LC_{50} was >2.60 mg/L in the rat. In one study, the rats showed breathing abnormalities after exposures at 2.6 mg/L for 4-hr. This value for the test substance is considered as potentially harmful for durations of exposure of the order of 4-hr. In two other studies, the mixture was shown to be not harmful at exposures up to 20 mg/L for 4-hr. Exposures via inhalation in these studies were via small droplets. Exposures via inhalation under field conditions will be smaller as the droplets are larger and less easily inhaled.
- The formulation is considered to be a slight and moderate irritant to the skin and eyes of the rabbit. The calculated primary irritation index for the test article was 0.25.

Based on these observations, the hazards to humans via application or bystander exposures are considered small and are limited to slight to moderate skin and eye irritation. These responses will be reduced if the affected areas are rinsed shortly after exposure to remove contamination. It was also concluded that the addition of the adjuvant Cosmo-Flux to glyphosate did not change the toxicological properties of the glyphosate formulation to mammals.

Effects of Formulated Glyphosate and Cosmo-Flux on Nontarget Aquatic Organisms

A base set of toxicity data is required for all pesticide registrations. For freshwater environments, the set normally makes use of a cold-water fish, such as rainbow trout fingerlings (*Onchorynchus mykiss*); a warm-water fish, such as fathead minnows (*Pimephales promelas*); an invertebrate such as the water flea (*Daphnia magna*); and an alga, such as *Selanastrum capricornutum*. Recognized guidelines were used for the tests (OECD 1984a,b, 1992; USEPA 1996b), which were conducted under the principles of GLP (OECD 1998b). These are standard test organisms, have been used for testing glyphosate itself and several other formulations, and thus are useful for comparison purposes. To reduce the requirement for animals in the testing, one combination of glyphosate and Cosmo-Flux, the combination for poppy (see Table 2), was selected. The results of these tests are summarized in Table 11.

C. Effects in the Field

Duration of Effects

In tropical forest situations, similar to some locations in coca eradication programs, there are limited data on vegetation recovery following glyphosate application. Nevertheless, there are a number of studies of successional patterns following land clearance and for tree gaps. Forest clearance has

Table 11. Toxicity values obtained from toxicity tests conducted on a mixture of glyphosate and Cosmo-Flux.

Test species	Common name	96-hr LC_{50}/EC_{50} in µg/L (as glyphosate AE)	Reference
Selenastrum	Algae, based on cell numbers, area under the growth curve, and growth rate	2,278–5,727[a]	Stantec (2005e)
Daphnia magna	Water flea, mortality	4,240 (3,230–5,720)[b]	Stantec (2005b)
Onchorynchus mykiss	Rainbow trout, mortality	1,847 (1,407–2,425)[b]	Stantec (2005d)
Pimephales promelas	Fathead minnow, mortality	4,600 (1,805–11,700)[b]	Stantec (2005c)

[a] Greatest and smallest effect measures in the study.
[b] LC_{50}/EC_{50} and 95% confidence Interval.

been a historical feature of the development of agriculture across the globe (Boahene 1998; Matlack 1997). In Central America, agricultural intensification and forest clearance in Mayan and other cultures has been determined from the pollen record (Clement and Horn 2001; Curtis et al. 1998; Goman and Byrne 1998). Patterns of successional change (recovery) in Neotropical forests have been reviewed by Gauriguata and Ostertag (2001). The authors noted that “the regenerative power of Neotropical forest vegetation is high, if propagule sources are close by and land use intensity before abandonment has not been severe.” However, they also caution that recovery is heavily dependent on interactions between site-specific factors and land use, “which make it extremely difficult to predict successional trajectories in anthropogenic settings.”

In relation to the eradication program, patterns of vegetation recovery will be dependent on size of plot, location of plot in relation to surrounding vegetation types, and local anthropogenic management, i.e., subsequent cultivation activities.

A study of tree regeneration in dry and humid selectively logged Bolivian tropical forests indicated that tree release with glyphosate in logging gaps had no significant impact on target tree species growth (Pariona et al. 2003). Although glyphosate controlled vegetation for a limited period, there were problems with the recruitment of commercial trees in logging gaps, suggesting a silvicultural need for site preparation treatments and more judicious seed tree retention.

Glyphosate has been widely used for controlling deciduous understory vegetation in managed northern forests, so-called conifer-release treatments (Lautenschlager and Sullivan 2002). Recovery of the deciduous herb and shrub layers occurs over 2–3 yr in general and the tree layer over 10 yr. Often, total structural diversity is unaffected by glyphosate treatments after 1 yr.

Forest Clearance and Soils. The impacts of forest clearance on soil fertility are generally well understood. Typically, tropical forest soils are fragile, being nutrient poor and subject to leaching. Tree clearance can quickly result in loss of nutrients, change in pH, and therefore change in element availability to plants (McAlister et al. 1998). Such conditions often allow only shifting cultivation under subsistence production, so-called slash-and-burn agriculture. Studies in Jamaican forests have shown that cultivations result in large amounts of soil erosion compared with secondary forest. An agroforestry treatment with *Calliandra calothyrsus* contour hedges reduced erosion and increased rainfall infiltration within the hedges (McDonald et al. 2002). As coca is a shrub, typically grown in rows, it might be argued that soil and water changes associated with forest clearance may be less than for annual crops such as maize, but clearly both have significant adverse effects on primary forest sites.

Although vegetation recovery may be rapid in eastern North America, research has led to the surprising conclusion that 19th-century agricultural practices decreased forest floor nutrient content and C:N and C:P ratios and increased nitrifier populations and net nitrate production, for approximately a century after abandonment (Compton and Boone 2000). The level of agricultural intensity, in terms of cultivation and fertilizer use, may have significant long-term impact on soils.

Effects on Associated Fauna. In an area of highly disturbed tropical dry forest in Cordoba Department, northern Colombia, small mammals were censused by live-trapping, running from secondary growth forest into agricultural areas (Adler et al. 1997). The results suggested the disturbed habitat supports a small mammal fauna of low diversity. However, several of the species appear to have benefited from forest clearance and agricultural activities and may occasionally reach extremely high numbers, although populations were not stable. A similar effect on reduced diversity of termites with increasing disturbance has been shown in dry forest in Uganda (Okwakol 2000). Changes in bird populations of a eucalypt forest in Australia following clear-felling indicate that full recovery may take up to 70 yr (Williams et al. 2001).

Although some species are adapted to disturbed conditions and can utilize agricultural land and secondary forest, there are many species associated with primary forest only, for example, the great argus pheasant in Indonesian tropical forests (Nijman 1998). With much of Colombia associated with extremely high biodiversity, there are very many endemic plant and animal species associated with national parks and in all likelihood with areas where coca and poppy are grown.

Studies on the impacts of vegetation change caused by glyphosate use on associated fauna in northern environments are available for some species. For example, following the application of glyphosate in clear-cut forest areas in Maine (USA), the use by moose (*Alces alces*) of treated and untreated areas was compared 1–2 yr and 7–11 yr postapplication (Eschenburg et al. 2003; Eschholz et al. 1996). At 1 and 2 yr posttreatment, tracks of foraging moose were 57% and 75% less abundant on treated than untreated clear-cuts ($P = 0.013$). However, at 7–11 yr posttreatment, tracks of foraging moose ($P = 0.05$) and moose beds ($P = 0.06$) were greater on treated than untreated clear-cuts. Less foraging activity at 1–2 yr posttreatment appeared to be the result of reduced browse availability, because conifer cover for bedding was similar on treated and untreated clear-cuts. The authors hypothesized that the greater counts of tracks of foraging moose on older treated clear-cuts were the result of increased foraging activity on sites with more abundant conifer cover (Eschholz et al. 1996; Raymond et al. 1996); i.e., tree cover had returned sufficiently after 10 yr. Studies of responses of small mammals to vegetation control with glyphosate in similar environments (Sullivan et al. 1998) indicated that vegetation recovery 2–3 yr after treatment was sufficient to return population dynamics to expected ranges.

Spot applications of glyphosate to reduce invasive ground flora in forests can have the beneficial effect of opening up the ground layer and encouraging spring ephemeral species to establish larger populations. Carlson and Gorchov (2004) reported this effect when controlling *Alliaria peteolata*, an invasive biennial plant. The impact of glyphosate on the target species was only for a single season.

Recovery from Effects. Glyphosate, as a well-translocated herbicide, affects most plant species if sufficient herbicide can penetrate plant tissues, particularly leaves. Effects typically result in plant death over 2–3 wk, although species with extensive storage organs, e.g., long rhizomes, large size, or particularly impenetrable leaf surfaces, may survive. A small dose of glyphosate can result in growth abnormalities in plants, most typically localized accelerated branching. If the dose of herbicide is insufficient to cause death, it has been proposed that plant fitness may also be reduced, such that if there is competition with other plants, death may result indirectly, though there is little published evidence for this.

The effect of glyphosate is limited to the plants that receive spray at the time of application, as the herbicide is rapidly adsorbed onto soil and root uptake does not occur. The broad spectrum of plant species controlled and the pattern of foliar uptake, together with the safety of the compound, have led to widespread use of the herbicide for total vegetation control, in preharvest weed control in annual crops, and for the control of perennial plants.

Recovery of treated areas is dependent on the initial level of control, the quantities of material (and the methods used) for plant regeneration and the environmental conditions of the site. Plants have a variety of adaptations for regenerating, with some life forms showing a range of methods, while others have only a single strategy. Monocarpic species, typically annuals, have seeds for recruitment of the next generation. Polycarpic species may also produce seeds, but many also have a variety of vegetative means of regenerating, such as rhizomes, bulbs, corms, and runners. Patterns of secondary succession, the resultant plant communities over time, reflect the plant–environment interactions and the opportunities for regeneration provided by the local species pool. Seeds in the soil or those that can reach a site from the surroundings, together with vegetative fragments, will establish initially. Continued agricultural operations, such as cutting or soil disturbance, will have a major influence on the species that survive. In most situations, vegetation recovery is rapid, with ruderal and pioneer plant species establishing within weeks of application.

In tropical forests, similar to some of the locations of the coca eradication programs, there are limited published data on vegetation recovery following glyphosate application. Nevertheless, there are a number of studies of successional patterns following land clearance and for tree gaps. Secondary succession (forest recovery) has become more common in some forest areas, for example, in Puerto Rico (Chinea 2002). Forest recovery is generally fairly rapid, but recovery of the full complement of forest species can take many years (>30 yr), and the effects of bulldozing for initial clearance can reduce diversity of native species and enhance establishment of non-native species. Comparisons of different aged plots (2–40 yr) in the Bolivian Amazon forests have contributed to the knowledge of secondary succession (Pena-Claros 2003). Not surprisingly, it takes longer for the forest canopy to achieve similar diversity to mature forest compared with the understory and subcanopy communities.

In relation to the eradication program, patterns of vegetation recovery will be dependent on size of plot, location of plot in relation to surrounding vegetation types, and local anthropogenic management, i.e., subsequent cultivation activities. Nevertheless, it should be noted that naturally occurring tree gaps (20–460 m^2) are an important component of overall forest diversity, providing opportunities for understory and subcanopy species and regeneration of canopy species in the modified light climate (Martins and Rodrigues 2002; Martins et al. 2004). In Brazilian varzea (white-water) forests, natural patterns of succession are affected by both light and local flooding (Wittmann et al. 2004). The patch scale of eradication applications of glyphosate may or may not be at the scale of natural forest gap dynamics; this deserves further study.

In the high Andes alpine paramo habitats, patterns of succession were described by Sarmiento et al. (2003). Following cultivation, usually for potato, patterns of secondary succession were such that, after 12 yr, species diversity of the undisturbed paramo had still not been attained. The characteristic paramo life forms, sclerophilous shrubs (e.g., *Baccharis prunifolia*, *Hypericum laricifolium*) and giant rosettes (e.g., *Espeletia schultzii*), appear very early and gradually increase in abundance during succession (Sarmiento et al. 2003).

In situations of agricultural expansion over large areas in Europe and North America, there is evidence that, where the proportion of remaining ancient habitat is small, subsequent forest recovery on abandoned agricultural land can be extended over long time periods (Vellend 2003). It is unlikely that habitat fragmentation and intensity of agriculture will combine to provide such a scenario in the coca eradication areas.

Effects on the successional patterns of vegetation in northern temperate and boreal forest situations are that woody and herbaceous species are most reduced by glyphosate (Bell et al. 1997). In a study in British Columbia, species richness, diversity, and turnover of the herb, shrub, and tree layers were not significantly ($P > 0.10$) different between mechanical and glyphosate spray cut stump treatments and a control. Similarly, the structural diversity of herb, shrub, and tree layers were also not significantly different ($P > 0.10$) between treatments and control. By opening the canopy and decreasing the dominance of the deciduous tree layer, both manual and cut-stump treatments showed greater total structural diversity (herb, shrub, and tree layers combined) relative to the control. However, differences in total structural diversity between treatments and control were, for the most part, not significant ($P > 0.10$). Therefore, these vegetation management treatments affected only the volume of the targeted deciduous tree layer and did not adversely affect species richness, diversity, turnover, or structural diversity of the plant community. These results may be applicable to other temperate forest ecosystems where conifer release is practiced in young plantations (Lindgren and Sullivan 2001). Herb biomass and cover usually recover to untreated values within 2–3 yr of conifer release treatment (Sullivan 1994). Meanwhile, the reduced competition on target conifers allows enhanced growth with little adverse effect on plant diversity (Sullivan et al. 1996, 1998).

Nevertheless, some plant groups may take longer to recover from glyphosate application. For example, cryptogams (ferns) may take longer than 5 yr to recover in boreal forest situations (Newmaster and Bell 2002), probably reflecting longer generation times and poor dispersal. Reviewing the effects of glyphosate use in forestry, Sullivan and Sullivan (2003) noted that single applications of glyphosate control much of the vegetation that receives spray, but recovery is generally rapid and within the range of natural disturbances.

Overall, the experience of glyphosate use in northern temperate forests is that vegetation and fauna recover over 2–3 yr following a single conifer-release treatment. With generally rapid plant growth under tropical conditions, available data confirm this scenario for Colombian conditions.

In comparison, land clearance for agriculture, or coca/poppy production, is a much more environmentally damaging operation, impacting adversely on soils in particular. Land clearance for illicit crops is already a threat to the conservation of bird species diversity in Colombia (Álvarez 2002). Although there are legitimate scientific questions as to the effects of (a) the spatial scale of individual glyphosate applications and (b) the return frequency of eradication treatments, field operational factors set these parameters. Spray areas reflect the patch scale of coca and poppy growing, averaging 1–2 ha each in a total of ~150,000 ha. Reapplication frequencies are generally greater than 6 mon for coca and greater than 3 mon for poppy and, bearing in mind the molecule is biologically unavailable in the soil and soil-bound residues have a half-life of 14–32 d, the environmental impacts are no greater than single applications.

V. Risk Assessment

The risk assessment was conducted by comparing estimated exposures to effect values for glyphosate from specific toxicity studies, from the literature, and from regulatory guidelines such as those established by the USEPA (1993b). The estimated exposures used were those calculated for the use of glyphosate for eradication spraying in Colombia.

A. Human Health

From an assessment of the results of toxicity testing of the formulation of glyphosate and Cosmo-Flux as used in Colombia, it was concluded that the addition of Cosmo-Flux to the spray mixture did not affect toxicity of the glyphosate to mammals. For this reason, it was possible to compare the toxicity of glyphosate and its formulations to exposures estimated under conditions of use in Colombia.

Exposures for the assessment were taken from Tables 5–7. The greatest values were taken as reasonable worst case for a hazard assessment. These results are shown in Table 12 and illustrated in Fig. 9. In comparing the exposure and effect concentrations, a margin of exposure approach was used. Thus a number greater than 1 (in Table 12) means that the exposure was less than criterion value or the exposure (or dose) that caused the response in the toxicology study. From these data, it is clear that potential exposures to glyphosate and Cosmo-Flux do not present a risk to human bystanders. In all cases, the margin of exposure for the most sensitive endpoint in laboratory animal studies with glyphosate was greater than 100, a conservative value often used to account for uncertainty in risk assessments of this type. As well, estimated worst case exposures were below the reference dose (RfD) established for glyphosate by the USEPA. The toxicity values used in both these approaches were derived from chronic exposures where the animals were dosed over extended time periods. They are thus

Table 12. Summary of reasonable worst case estimated exposures of humans to glyphosate resulting from use in the eradication of coca and poppy in Colombia and margins of exposure.

	Exposure value (mg/kg)		Margin of exposure compared to the most sensitive NOEL (175 mg/kg bw)	
Source of exposure	Coca	Poppy	Coca	Poppy
Direct overspray	0.04	0.01	4,918	20,417
Reentry	0.26	0.06	676	2,804
Inhalation	0.01	0.01	28,226	28,226
Diet and water	0.75	0.18	234	972
Worst case total exposure from all sources	1.05	0.26	167	680

	Exposure value (mg/kg)		Margin of exposure for the U.S. EPA RfD (2 mg/kg bw/d)	
Source of exposure	Coca	Poppy	Coca	Poppy
Direct overspray	0.04	0.01	56	233
Reentry	0.26	0.06	8	32
Inhalation	0.01	0.01	323	323
Diet and water	0.75	0.18	2.7	11.1
Worst case total exposure from all sources	1.05	0.26	1.9	7.8

additionally protective of short and infrequent exposures that would occur during the use of glyphosate in the eradication spray program. Some exposure values were close to the inhalation toxicity value, but as already discussed, droplet size is large and inhalation will be less than in the laboratory animal studies as well as the droplet size used in agriculture, from which the potential inhalation exposure was derived.

B. Environment

The acute toxicity data for formulated glyphosate in aquatic animals from Solomon and Thompson (2003) were combined with some of the new data for amphibians described above and are displayed graphically as a point of reference for characterizing the toxicity of glyphosate plus Cosmo-Flux as

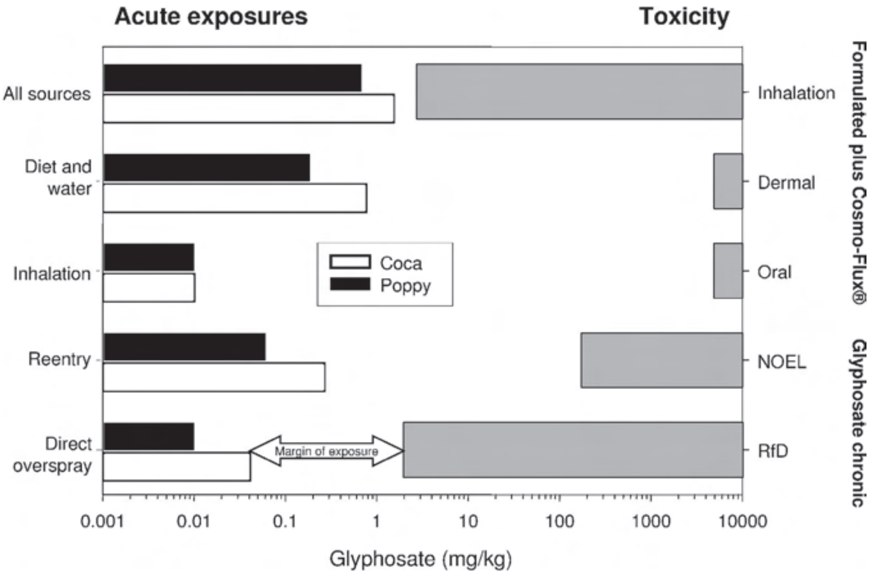


Fig. 9. Illustration of acute toxicity values in laboratory mammals for glyphosate plus Cosmo-Flux, the no-observed-effect-level (NOEL) from the most sensitive chronic study in laboratory animals, and the reference dose (RfD) (glyphosate) and the estimated worst case acute exposures that may be experienced under conditions of use in Colombia.

used in Colombia (Fig. 10). The graph is presented as a cumulative frequency distribution in a manner similar to that used in probabilistic risk assessments for pesticides (Solomon and Takacs 2002). The combination of formulated glyphosate and Cosmo-Flux, as used in Colombia, is more toxic to the aquatic organisms tested than formulations of glyphosate without the addition of surfactants and/or adjuvants, which is not altogether surprising. The toxicity of glyphosate itself to aquatic organisms is very small (Solomon and Thompson 2003) but, when mixed with some surfactants and adjuvants, this toxicity can be increased. The toxicity of Cosmo-Flux was not tested on its own; however, from experience with other adjuvants, it clearly contributes to the increased toxicity of the mixture. It is interesting to note that larval amphibians appear to be more susceptible to glyphosate formulations than other aquatic animals. The reason for this is likely the surfactants in the formulation of Roundup, as already discussed, as other formulations of glyphosate are less toxic to amphibians (Howe et al. 2004).

Assessment of the environmental risks of glyphosate and Cosmo-Flux to aquatic organisms was based on toxicity data from the literature and from studies conducted on the mixture of formulated glyphosate and Cosmo-Flux as used in Colombia. When the toxicity values for the mixture as used in Colombia are compared with the range of estimated exposures that would result from a direct overspray of surface waters (see Table 8), it is clear that aquatic animals and algae in some shallow water bodies may be at risk (see Fig. 10).

Although the overlap of the range of estimated exposure concentrations with the toxicity values for green alga and rainbow trout suggest that there may be increased risk in situations where an accidental overspray will occur, this would have to be in a location where a shallow water body is close enough to the coca field that it is accidentally oversprayed, that it is less than 30 cm deep, and that it is not flowing. Water flow would likely result in rapid hydraulic dilution to concentrations below the threshold of biological activity, so organisms in flowing water would not be at great risk. It was not possible to determine the actual frequency of these risks, as data on proximity of surface water to coca fields are not available. Based on the toxicity data with formulated Roundup in amphibians, this group of organisms may be at risk; however, specific testing in amphibians has not been conducted on the glyphosate plus Cosmo-Flux as used in Colombia.

Based on the toxicity data for honeybees, glyphosate and Cosmo-Flux is not acutely toxic via contact exposure to honeybees. It caused no mortality

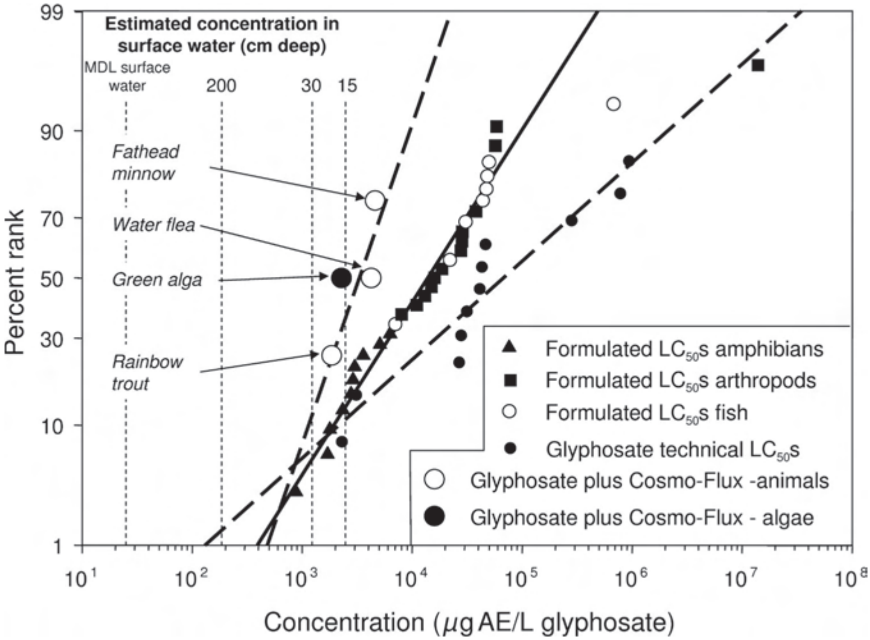


Fig. 10. Cumulative frequency distribution of toxicity values for glyphosate technical, formulated glyphosate (Roundup) in all aquatic organisms and in fish and the toxicity values in four aquatic species for glyphosate and Cosmo-Flux 411 mixture as used in Colombia.

or stress effects in bees in the normal 48-hr period after treatment at concentrations equal to or less than 63.9 mg AE/bee, showing that the formulated product is not directly hazardous to bees or, by extrapolation, to other beneficial insects.

Although no acute or chronic data are available on wild animals, extrapolation of the mammalian data discussed earlier and reports in the literature support the conclusion that glyphosate and Cosmo-Flux will not have adverse direct effects on wild mammals or birds. Indirect effects through habitat alteration are possible. However, it is unlikely that the coca and poppy fields are significant habitats for wildlife. Human activities related to cultivation, pest control, and harvesting will be more disruptive to wildlife, and death of the coca bushes or the poppy plants as a result of spraying with glyphosate will not add an additional stressor. In fact, if the sprayed area is not replanted and allowed to naturalize, this new successional habitat may be more attractive to birds and mammals than an old-growth forest. Given that coca and poppy fields are usually located in remote areas and are often surrounded by natural habitats, sources for recolonization or alternate habitats will be close by. Some habitat alteration will result from accidental oversprays that affect nontarget vegetation; however, as already discussed, these areas are small in relation to the sprayed fields (<0.48%), represent a very small proportion of the total habitat available ($\ll$0.001%), and will undergo rapid recolonization and succession to habitats suitable for wildlife.

VI. Conclusions

Risks associated with the use of glyphosate and Cosmo-Flux in the coca and poppy eradication program in Colombia are related to the total impacts of coca and poppy production discussed in the section on Problem Formulation. There are a number of other activities associated with the production of cocaine and heroin that result in risks to human health and the environment. Data are not available to quantify all these risks, but some of them may be estimated on the basis of other knowledge and expert judgment, which was done using an adaptation of a risk prioritization scheme used in ecological risk assessment (Harwell et al. 1992).

For purposes of ranking human health hazards, the intensity score ranged from 0 to 5, with 5 being a severe effect such as a physical injury or toxicity. The recovery score also ranged from 0 to 5 and was based on the potential for complete recovery from the adverse effect. Frequency was based on an estimate of the proportion (%) of the total number of persons involved in coca and poppy cultivation, production, and the refinement of cocaine and heroin. The score for impact was the product of the individual scores and the percent impact is based on the sum of the impact scores.

A similar procedure to that described above was used for ranking ecological risks associated with the cycle of coca and poppy production.

The intensity score was ranked from 0 to 5, with 5 being most intense, such as the total destruction of the habitat by clear-cutting and burning a natural area. Intensity of effects in this case also included off-field effects such as on nontarget animals and plants. Recovery time in this scheme is the estimated time for the impacted area to recover to a state similar to the initial condition. In the case of clear-cutting and burning, it is recognized that succession will begin immediately; however, full recovery to a mature and diverse tropical forest may take considerably more than the 60 yr estimated here. Similarly, in the absence of cultivation, it was estimated that invasive and competitive species will displace coca and poppy in several years, and an estimate of 4 yr was used in this case. Given the need to apply fertilizer and pesticides frequently because of utilization of nutrients and resurgence of pests, the recovery time for these ecological impacts was judged to be small. The scores were multiplied to give the impact score, and the percent impact was based on the sum of the impact scores.

A. Human Health Relevance

Based on all the evidence and information presented here, we concluded that the risks to humans and human health from the use of glyphosate and Cosmo-Flux in the control of coca and poppy were minimal (Table 13). Acute toxicity of the formulated product and Cosmo-Flux to laboratory animals was very low, the likely exposures were low, and the frequency of exposures was low. When these risks are compared with other risks associated with clearing of land, the uncontrolled and unmonitored use of other pesticides (many of them more toxic to humans than glyphosate, CICAD/

Table 13. Potential human health impacts of the cycle of coca or poppy production in the Colombian environment.

Impacts	Intensity score	Recovery score	Frequency (%)	Impact score	Impact (%)
Clear cutting and burning	5	3	3	45	16.7
Planting the coca or poppy	0	1	100	0	0.0
Fertilizer inputs	0	0.5	10	0	0.0
Pesticide inputs	5	3	10	150	55.6
Eradication spray	0	0	10	0	0.0
Processing and refining	5	3	5	75	27.8

OAS 2005) to protect the coca and poppy, and exposures to substances used in the refining of the raw product into cocaine and heroin, they are essentially negligible.

B. Ecological Relevance

Based on evidence and data discussed above and results of a number of specific studies conducted specifically for this assessment, we concluded that the risks to the environment from the use of glyphosate and Cosmo-Flux in the control of coca and poppy were small in most circumstances (Table 14). Risks of direct effects in terrestrial wildlife such as mammals and birds were judged to be negligible, as were those to beneficial insects such as bees. Moderate risks to some aquatic wildlife may exist in some locations where shallow and static water bodies are located in close proximity to coca fields and are accidentally oversprayed. However, when taken in the context of the environmental risks from other activities associated with the production of coca and poppy, in particular, the uncontrolled and unplanned clearing of pristine lands in ecologically important areas for the purposes of planting the crop, the added risks associated with the spray program are small.

Table 14. Potential environmental impacts of the coca or poppy production cycle in the Colombian environment.

Impacts	Intensity score	Recovery time (years)	Impact score	Impact%
Clear cutting and burning	5	60	300	96.9
Planting the coca or poppy	1	4	4	1.3
Fertilizer inputs	1	0.5	0.5	0.2
Pesticide inputs	5	0.5	2.5	0.8
Eradication spray	1	0.5	0.5	0.2
Processing and refining	2	1	2	0.6

C. Strengths and Uncertainties in the Assessment

This assessment has both strengths and uncertainties, as discussed in the following sections; these lie in the exposure and effects characterizations and, because they are used in the risk characterization, are also reflected in the risk assessment. Uncertainties are inherent in all risk assessments and, in some cases, can be easily addressed though additional data collection or specific studies. Recommendations for additional studies and data collection are addressed in the final section.

Exposures

Human Exposures. Human exposures to glyphosate were estimated from extensive and well-documented studies in other jurisdictions and are judged to be accurate with respect to bystanders who are directly oversprayed. Exposures were judged to be small and, in all cases, considerably below thresholds of concern.

Application rates of glyphosate used for coca control are greater than those used in conventional agriculture, suggesting that experience and exposures measured under these conditions may not be applicable to bystander exposures in eradication spraying in Colombia. While this may be true, the margins between exposures doses at which chronic effects may occur are great enough to provide a wide margin of safety to bystanders. Less information is available regarding the likelihood of exposure upon reentry to coca fields immediately after spraying; this relates to the anecdotal evidence that picking of leaves or pruning of plants immediately after they are sprayed with glyphosate will "save" the plants. Exposures under these conditions are unmeasured, but are estimated to be below the USEPA reference dose.

Environmental Exposures. Applications of glyphosate are well characterized using state-of-the-art equipment. Locations of application and areas sprayed are well documented and measured with resolutions only equaled in some applications in forestry in other jurisdictions. Mixing and application rates are well characterized, and the probability of application of amounts of glyphosate and Cosmo-Flux greater than those specified is small. Concentrations in soil and water that may result from an accidental overspray also have high certainty. The environmental behavior of glyphosate is well characterized and, under the conditions of use, it will not persist, accumulate, or biomagnify. Analyses of surface waters and sediments in one watershed where eradication spraying was carried out did not reveal the presence of significant concentrations of glyphosate, confirming the conclusion based on its properties that it is not mobile in the environment. Residues of glyphosate were infrequently detected in areas where eradication spraying was not conducted but where glyphosate use was known to occur in agriculture. Given that considerably more glyphosate is used in agriculture and other noneradication uses (~85%), this further confirms that glyphosate is not sufficiently mobile to result in significant contamination of surface waters in Colombia, regardless of the use pattern.

Uncertainties in the exposure characterization lie in lack of precise measurements of the proximity of sprayed fields to surface waters and the proportion of treated areas that are in close proximity. Sampling of the surface waters only took place for 24 wk, and only five locations were sampled. Although two of these were scheduled to be sprayed, only one was treated during the sampling period. For logistical reasons, it was also not possible to sample close to application sites. Had sampling been conducted at more sites closer to the sprayed fields and over a longer time period, residues may have been detected more frequently.

Effects

Effects in Humans. The database of glyphosate effects is large and its risks to humans and the environment have been extensively reviewed and assessed in a number of national and international jurisdictions as well as in the open scientific literature. In all cases, glyphosate poses little risk. However, some of the studies on which these assessments are based were conducted before the refinement of testing guidelines and the availability of new and more sensitive methods of analysis and effect characterization, such as those based on alteration in the concentrations of neurotransmitters and their metabolites in the central nervous system. In the process of reassessment and reregistration, older studies will be replaced with newer tests using current guidelines. Given the large and expanding use of glyphosate in agriculture, priorities for updating the database will likely be high. Changes in the regulatory status of glyphosate should be monitored and any newly identified risks included in an updated risk assessment.

There is considerable literature on the epidemiology of pesticides and possible effects on human health. As a result of recent work, it is clear that many epidemiology studies are confounded by the use of poor and inaccurate surrogates for exposures to pesticides. We also conducted a preliminary epidemiological study to assess possible linkages between the use of glyphosate and adverse human health outcomes; this study recognizes that, for clear logistical reasons, no measures of exposure were available for the various groups enrolled in the study, other than the use of glyphosate for eradication spraying in the region. The results do not suggest that there is an association between the use of glyphosate in the eradication program and time to pregnancy (TTP) as a reproductive outcome. A somewhat greater risk for longer TTP was observed in one region (Valle del Cauca) where eradication spraying is not conducted, but it was not possible to identify any specific factors that may have been responsible for this observation.

Environmental Effects. The environmental toxicology database for glyphosate is relatively large, and its effects in nontarget organisms are well known or can be extrapolated. Glyphosate itself is essentially nontoxic to nontarget organisms. However, there are a number of formulations of glyphosate in the marketplace that contain many different surfactants and/or adjuvants. It is also known that it is the surfactants that determine the toxicity of the formulation and are more toxic than technical glyphosate itself. Because of this, several toxicity tests were conducted with the formulated product of glyphosate plus Cosmo-Flux used in the Colombian program; this reduced uncertainty with respect to toxicity to beneficial insects, such as the honeybee, and to aquatic organisms. Recent studies have reported that frogs are among the more-sensitive aquatic organisms to formulations of glyphosate such as Roundup and Vision. We did not conduct toxicity studies in amphibians with the mixture of glyphosate plus Cosmo-Flux, and this is a source of some uncertainty for ecological risks for frogs.

Confounding Risks

Through the Tier 1 and Tier 2 hazard assessments of the other substances used in the production and refining of cocaine and heroin (CICAD/OAS 2004c, 2005), we recognize that some of these substances present a significantly greater hazard to both humans and the environment than does the mixture of glyphosate and Cosmo-Flux used in the program. Exacerbating these hazards is the lack of information about the conditions of their use. Because of the lack of specific data on use and exposure, it was not possible to conduct detailed risk assessments for these substances. From anecdotal evidence and observations in other locations, it is clear that, in most cases, these substances are used without adequate safety training, without adequate protective equipment, without suitable disposal methods, and without supervision, which represents a significant and serious potential risk to humans and the environment.

D. Recommendations

We have identified a number of uncertainties in our review of the data and, from these, make the following recommendations. These recommendations are grouped into two classes, recommendations to retain current practices that were judged to be essential or useful (Table 15) and recommendations related to new activities or data collection which will address key uncertainties identified in our study (Table 16). As already noted, risk assessments require review and reevaluation from time to time. Thus, our recommendations include the updating of this risk assessment as additional data become available.

Summary

The production of coca and poppy as well as the processing and production of cocaine and heroin involve significant environmental impacts. Both coca and poppy are grown intensively in a process that involves the clearing of land in remote areas, the planting of the crop, and protection against pests such as weeds, insects, and pathogens. The aerial spray program to control coca and poppy production in Colombia with the herbicide glyphosate is

Table 15. Recommendations for the continuance of current practices in the coca and poppy eradication program in Colombia.

Practice	Benefit of continuance	Ranking of importance (5 = most important)
Mixer-loader, worker, and environmental protection in the storage, mixing, and loading operations.	Protection of the humans and the environment from excessive exposures.	5
Use of state-of-art application technology.	Accurate records of location and areas sprayed.	5
Replace the respirator worn by the mixer-loader with a full face shield to reduce the potential for splashed material to run down the face into the eyes.	This recommendation is procedures modification of current that will reduce the risk of splashes of concentrated formulation into the eyes.	5
Use of glyphosate in the eradication program.	The risk of this product to humans and the environment is judged to be smaller than any currently available alternatives. However, if new candidate products become available, their use should be considered only after an appropriate risk assessment has been conducted.	4

conducted with modern state-of-the-art aircraft and spray equipment. As a result of the use of best available spray and navigation technology, the likelihood of accidental off-target spraying is small and is estimated to be less than 1% of the total area sprayed.

Estimated exposures in humans resulting from direct overspray, contact with treated foliage after reentry to fields, inhalation, diet, and drinking water were small and infrequent. Analyses of surface waters in five watersheds showed that, on most occasions, glyphosate was not present at measurable concentrations; only two samples had residues just above the method detection limit of 25 µg/L. Concentrations of glyphosate in air were predicted to be very small because of negligible volatility. Glyphosate in soils that are directly sprayed will be tightly bound and biologically unavailable and have no residual activity. Concentrations of glyphosate plus Cosmo-Flux will be relatively large in shallow surface waters that are directly

Table 16. Recommendations for the collection of new data and information in the coca and poppy eradication program in Colombia.

Recommendation	Benefit of new data	Ranking of importance (5 = most important)
Conduct a study to identify risk factors associated with time to pregnancy (TTP).	This is a recommendation resulting from the observation of increased risk of longer TTP in one region of Colombia (Valle del Cauca) where eradication spraying was not carried out. The study should be considered for prioritization in the general human health research programs conducted in Colombia.	3
Including proximity to surface waters in Geographic Information System (GIS) analysis of locations and areas of coca and poppy fields.	Better indication of likely frequency of contamination of these habitats; this would help to better quantify the risks to aquatic organisms in shallow-water nonflowing habitats.	2
Identify mixtures of glyphosate and adjuvants that are less toxic to aquatic organisms than the currently used mixture. The priority of this recommendation would depend on the results of the GIS analysis.	Reduction in possible environmental impacts to nontarget organisms in shallow surface water environments.	2
Testing of the glyphosate-Cosmo-Flux formulation for toxicity to amphibians.	Decrease in uncertainty regarding the toxicity to amphibians.	2
Use of GIS to quantify areas of coca and poppy production in biodiversity hotspots.	Better quantification of proportion of regions identified as important sources of biodiversity that are being adversely impacted because of clear-cutting and planting of coca and poppy.	2

Table 16. *Continued*

Recommendation	Benefit of new data	Ranking of importance (5 = most important)
Use of GIS to quantify size of fields planted to coca and poppy and track these over time to judge extent of environmental impact as well as recovery.	Allow more-accurate quantification of potentially impacted areas as well as recovery when these fields are abandoned.	2
Review the regulatory status of glyphosate on a regular basis.	Ensure that new testing and toxicity data on glyphosate are included in the risk assessment of its use in eradication spraying in Colombia.	2
Measurement of exposures to glyphosate in bystanders to sprays and reentry into sprayed fields. This recommendation would follow selection of new formulations and mixtures of adjuvants that have less environmental toxicity.	Better characterization of exposures under conditions of use in Colombia.	1

oversprayed (maximum instantaneous concentration of 1,229 μg AE/L in water 30 cm deep); however, no information was available on the number of fields in close proximity to surface waters, and thus it was not possible to estimate the likelihood of such contamination.

The formulation used in Colombia, a mixture of glyphosate and Cosmo-Flux, has low toxicity to mammals by all routes of exposure, although some temporary eye irritation may occur. Published epidemiological studies have not suggested a strong or consistent linkage between glyphosate use and specific human health outcomes. An epidemiology study conducted in Colombia did not show any association between time to pregnancy in humans and the use of glyphosate in eradication spraying.

The mixture of glyphosate and Cosmo-Flux was not toxic to honeybees. The mixture was, however, more toxic to the alga *Selenastrum*, the cladoceran *Daphnia magna*, fathead minnow, and rainbow trout than formulated glyphosate (Roundup) alone. Studies on the use of glyphosate in agriculture and forestry have shown that direct effects on nontarget organisms other than plants are unlikely. Indirect effects on terrestrial arthropods and other wildlife may be the result of habitat alteration and environmental change brought about by the removal of plants by glyphosate. Because of the lack of residual activity, recovery of glyphosate-treated areas in Colombia is expected to be rapid because of good plant growth conditions. However, return to the conditions of tropical old-growth forest that existed before clear-cutting and burning may take hundreds of years, not from the use of glyphosate but because of the clear-cutting and burning, which are the primary cause of effects in the environment.

The risk assessment concluded that glyphosate and Cosmo-Flux did not present a significant risk to human health. In the entire cycle of coca and poppy production and eradication, human health risks associated with physical injury during clear-cutting and burning and the use of pesticides for protection of the illicit crops were judged to be considerably more important than those from exposure to glyphosate. For the environment, direct risks from the use of glyphosate and Cosmo-Flux to terrestrial mammals and birds were judged to be negligible. Moderate risks could occur in aquatic organisms in shallow surface waters that are oversprayed during the eradication program. However, the frequency of occurrence and extent to which this happens are unknown as data on the proximity of surface waters to coca fields were not available. Considering the effects of the entire cycle of coca and poppy production and eradication, clear-cutting and burning and displacement of the natural flora and fauna were identified as the greatest environmental risks and are considerably more important than those from the use of glyphosate for the control of coca and poppy.

Acknowledgments

This review is based on a report (Solomon et al. 2005a) prepared for the Inter-American Drug Abuse Control Commission (CICAD) section of the Organization of American States (OAS) in response to requests from the Governments of Colombia, the United Kingdom, and the United States. The request was to conduct a science-based risk assessment of the human health and environmental effects of the herbicide, glyphosate, used for the control of the illicit crops coca and poppy in Colombia.

With an international panel of experts and activities in several countries, a study of this nature requires good coordination and organization. We are deeply indebted to Mr. Jorge Rios and Ms. Adriana Henao of the CICAD office for their excellent work in organizing meetings, teleconferences, and field trips. They served the authors well and frequently worked well beyond the call of duty. We are also very grateful for the contributions of the Colombian Field Team. Unfortunately, we cannot name these individuals; however, we extend our most grateful thanks to all of you for all the hard work and the personal risks that you took on behalf of data collection for this project.

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