ENVIRONMENTAL AND HUMAN HEALTH ASSESSMENT OF THE AERIAL SPRAY PROGRAM FOR COCA AND POPPY CONTROL IN COLOMBIA

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PREFACE

This report was 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 of America. 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.

The initial step in the process was to establish an international panel of experts in human and environmental toxicology, in epidemiology, in agronomic practices, and in ecology (SAT). Because both Colombia and the United States were actively involved in the program for eradication of illicit crops, members of the panel were specifically selected from other countries.

Initially, the panel met to formulate a framework to conduct this risk assessment. The framework was based on those commonly used for risk assessment in a number of jurisdictions and consisted of a problem formulation, characterization of the human health and environmental effects of the substances used in the eradication program, characterization of human and environmental exposures, and the drawing together of these in a risk characterization. During this process, extensive use was made of the scientific and other literature but, where data gaps and uncertainties related to the specific uses in Colombia were identified, studies were initiated to assemble additional data for use in the risk assessment. Some of these studies were carried out in Colombia. The Colombian team (PTG) were contracted specifically to CICAD and worked under the direction of the SAT to collect data in the Colombian Environment. During the conduct of our study, members of the SAT made a number of visits to Colombia to view, at first hand, all aspects of the program, to gather local information and data, and to oversee the local studies of the PTG.

We recognize that the illicit crop eradication program in Colombia has generated considerable local and international interest and is the subject of intense debate for political, social, and other reasons. We have specifically excluded all social, political, and economic issues from our study and the final report is strictly based in science and scientifically based arguments. We believe that the report of the study and its scientific recommendations will be useful in decision making to protect human health and the environment.

After the initiation of this project, additional information on other substances used in the production of coca and poppy and the refining of cocaine and heroin was requested. This request culminated in two separate detailed reports, a Tier-1 and Tier-2 hazard assessment of 67 and 20 substances used for these purposes, respectively. These substances are briefly discussed in the Problem Formulation of this report. We believe that these reports will be useful in comparative hazard assessment and in risk management decision making.

ACKNOWLEDGEMENTS

With an international panel of experts and activities in several countries, a study of this nature requires good co-ordination 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 Panel well and frequently worked well beyond the call of duty. We are also very grateful for the contributions of the Colombian Field Team, the PTG. 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.

Field visits to Colombia by members of the SAT were facilitated and coordinated by the staff of the Ministry of Foreign Affairs and the team was afforded protection by the National Police (Antinarcoticos). We offer our grateful thanks to Brigadier General Luis Gómez, his staff, the pilots, technicians, and the "Junglas" commandos for aiding us in our observations and sampling and for tolerating our scientific curiosity in the face of other priorities. At all times, we were given free and unfettered access to information, we were allowed to take photographs freely, and we were always treated with respect and in a most professional manner.

The SAT members are indebted to Drs. Lesbia Smith, Angus Crossan, Richard Brain, and also to the many students in the Toxicology Program at the University of Guelph for their work on the separate reports on Tier-1 and Tier-2 hazard assessment of other substances used in the production and refining of cocaine and heroin. These data are presented in separate reports.

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EXECUTIVE SUMMARY

This report was 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 States, and the United Kingdom. The request was to conduct a science-based risk assessment of the human health and environmental effects of the use of glyphosate for the control of the illicit crops, coca and poppy, in Colombia. This became the purpose of the study, which was conducted in a number of steps.

The initial step in the process was to establish an international Panel of experts in human, animal, and environmental toxicology, in epidemiology, in agronomic practices, and in ecology (the Scientific Advisory Team - SAT). In the second step, the SAT formulated a framework to conduct this risk assessment. The framework is similar to those commonly used for assessing risks in a number of jurisdictions and consisted of a problem formulation, characterization of the human health and environmental effects of the substances used in the eradication program, characterization of human and environmental exposures, and the drawing together of these in a risk characterization. During the process of conducting the risk assessment, the SAT used scientific literature and government reports but, where data gaps and uncertainties related to the specific uses in Colombia were identified, studies were initiated to assemble additional data for use in the risk assessment. Several of these studies were carried out in Colombia. The Colombian Team (PTG) were contracted specifically to CICAD and worked under the direction of the SAT to collect data in the Colombian environment. During the conduct of our study, members of the SAT made a number of visits to Colombia to view, at first hand, all aspects of the program, to gather local information and data, and to oversee the local studies of the PTG.

The SAT recognized that the growing and production of illicit drugs in Colombia has significant political, social, and economic, implications. However, this study was focused specifically on the human health and environmental significance of the production and eradication of coca and poppy through the use of aerially applied herbicide. The production of coca and poppy as well as the processing and production of cocaine and heroin also involves 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 protection against pests such as weeds, insects, and pathogens. All of these activities can impact human health and the environment and some, such as clear-cutting, do so to a significant extent. The total land area used for these activities is small relative to the entire country. However, much of the production takes place in remote areas that are close to or part of the Andean Biodiversity Hotspot.

In Colombia, the herbicide glyphosate is widely used in agriculture and for purposes other than eradication of coca and poppy. Only 10-14% of the total use in Colombia is in the eradication program. Similarly many of the pesticides and other substances used in the production of coca and poppy are also widely used in agriculture. The aerial eradication spray program in Colombia is conducted with modern state-of-the-art aircraft and spray equipment. The spray equipment is similar to that used for forest spraying in other parts of the world and produces large droplets which minimize drift of spray. Identification of target fields and electronic documentation

of locations and areas sprayed is conducted with high precision. 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.

The physical, chemical, and biological properties of glyphosate and an adjuvant (Cosmo-Flux®) added to the spray mix were characterized through the scientific literature and through new studies specifically conducted for this risk assessment. Glyphosate is a widely-used herbicide that is well characterized in terms of physical, chemical, and biological properties. Glyphosate is not highly mobile in the environment and is rapidly and tightly bound on contact with soil and aquatic sediments. Glyphosate has a very short biological activity in soils and water, does not biomagnify or move through the food chain, and does not leach into groundwater from soil.

Exposures of humans to glyphosate under the conditions of use could not be measured directly in the growers of illicit crops and thus were estimated from literature values with adjustments for the rates of application used in the eradication program in Colombia. Estimated exposures resulting from direct overspray, contact with treated foliage after re-entry to fields, inhalation, diet, and drinking water were small and infrequent. In a special study in five watersheds, weekly analyses of surface waters and sediments over a period of 24 weeks showed that, on most occasions, glyphosate was not present at measurable concentrations; only two samples had residues above the method detection limit of 25 µg/L. As most of the glyphosate used in Colombia is in agriculture, this confirms that, regardless of use pattern, glyphosate is not mobile in environment and it will not move from the treated fields in significant amounts. In analyses of water samples taken from the same five watersheds, several other pesticides were found, including the herbicide 2,4-D and the insecticide endosulfan, the latter a product that is banned in Colombia.

Concentrations of glyphosate in several environmental matrices resulting from the eradication spray program were estimated. Concentrations 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. Based on observations in other temperate and tropical areas, no residual activity is expected in soil and even the most sensitive organisms, plants, will not be prevented from re-establishing themselves. In Colombia, this is evidenced by the rapid recovery of sprayed fields through successful replanting of coca and/or colonization by invasive species of plants. Concentrations of glyphosate plus Cosmo-Flux® will be relatively large in shallow surface waters that are over-sprayed (maximum instantaneous concentration of 1,052 µg AE/L in water 300 mm deep); however, no information was available on the number of fields in close proximity to surface waters and it was not possible to estimate the likelihood of such contamination.

The toxicity of glyphosate has been rigorously assessed in a number of jurisdictions and in the published literature. Glyphosate itself has low toxicity to non-target organisms other than green plants. It is judged to have low acute and chronic toxicity, carcinogenic, mutagenic, or a reproductive toxicant. With respect to humans, is not considered hazardous, except for the possibility of eye and possibly skin irritation (from which recovery occurs). The toxicity of the formulation as used in the eradication program in Colombia, a mixture of glyphosate and Cosmo-Flux®, has been characterized in specific tests conducted in laboratory animals. The mixture has low toxicity to mammals by all routes of exposure, although some temporary eye irritation may occur. By extrapolation, the spray mixture is also not expected to be toxic to terrestrial mammals and vertebrates. Epidemiology studies conducted in a number of jurisdictions around the world have not suggested a strong or consistent linkage between glyphosate use and specific human health outcomes. A preliminary epidemiology study was conducted in Colombia to assess any linkage between glyphosate and the reproductive outcome, time to pregnancy, in humans. This study did not show any association between time to pregnancy and the use of glyphosate in eradication spraying.

New data from the environmental literature on the toxicity of some formulations of glyphosate suggest that amphibians may be the most sensitive group of aquatic organisms. Special tests of the spray mixture as used in Colombia were conducted using standardized environmental test organisms. These tests revealed that the mixture of glyphosate and Cosmo-Flux® was not toxic to honey bees. The mixture was, however, more toxic to aquatic organisms than formulated glyphosate alone. Extensive studies on the use of glyphosate in agriculture and forestry in temperate and tropical areas have been published in the literature. These have shown that direct effects on non-target organisms other than plants are unlikely to occur. Indirect effects on terrestrial arthropods and other wildlife have, however, been observed. These are the result of habitat alteration and environmental change brought about by the removal of target plants through the effects of glyphosate. Similar effects would be expected regardless of the type of method used to control plants and also occur as a result of clear-cutting, burning, and conversion of natural areas into agricultural lands. Because of the lack of residual activity, recovery of glyphosate-treated areas will be dependent only on the nature of the recolonizing species and the local conditions. Given experience in other tropical regions and in Colombia, this process will be rapid because of good conditions for plant growth. However, return to the conditions of tropical old-growth forest that existed prior to clear-cutting and burning may take hundreds of years. It is important to recognize that the impact here is not the use of glyphosate but the original act of clear-cutting and burning that is the primary cause of the effects on the environment.

The risk assessment concluded that glyphosate and Cosmo-Flux® as used in the eradication program in Colombia did not present a significant risk to human health. Estimated acute worst-case exposures in humans via all routes were less than doses of concern, even for chronic responses. 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 more important than those from exposure to glyphosate.

For the environment, risks from the use of glyphosate and Cosmo-Flux® to terrestrial animals were judged to be small to negligible. Moderate risks could occur in aquatic organisms in shallow surface waters that are over-sprayed 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.

Strengths and uncertainties in the assessment were identified and used to develop recommendations which were then prioritized. It is recommended that the current application practices for eradication spraying be retained but that additional data be gathered over a longer time period to better characterize the impacts of coca and poppy production in the Andean Biodiversity Hotspot and the possibility of non-target effects in surface waters located close to fields. If shallow waters are routinely found close to fields, it is recommended that other formulants be tested for the purposes of selecting products that present a lower risk to aquatic organisms. Although no association was observed between eradication spraying and reproductive outcomes in humans, additional studies to identify possible risk factors associated with other human activities or environmental factors should be considered.

1 INTRODUCTION

1.1 BACKGROUND

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 (United Nations 2002). In response to the socio-economic 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 production and distribution (United Nations 2002). While it is recognized that the political, social, and economic impacts of the production, distribution, and use of all of these drugs is significant, the focus of this report is on issues related to the program for reduction and eradication of production of coca and opium poppy and their derivatives, cocaine and the opiates in Colombia, South America.

Coca (*Erythroxylum coca* and related species, Figure 1) are commonly associated with the tropical mountainous regions of South America. However, it has been reported to be grown in Africa, Sri Lanka, Taiwan, and Indonesia (Bray and Dallery 1983). 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 tropical regions with temperatures above 25°C and moderate to high rainfall >1000 mm per year. Currently, it is widely cultivated in Colombia, Bolivia, and Peru, with some cultivation in Ecuador, Venezuela, Brazil, and Argentina as well.

Figure 8 Coca plant

Historically, coca played an important role in culture of the Incas, Quechuas, and many other Andean peoples. Coca also played an important role in the conquest of Latin America by the Spanish when it was used as an incentive and payment for work on railroads, in agriculture, and in mines. More recently, cocaine, derived from the coca plant, has become widely used in many countries. Initially used as a medicinal drug, it was introduced to Europe as cocaine in 1860 as an ingredient of a wine-coca drink which was apparently used by the likes of Sarah Bernhardt, Queen Victoria of England, Thomas Edison, and Pope Leo the XIII. It was also used as a local anesthetic. In 1886, John Pemberton introduced the tonic drink CocaCola® which contained cocaine until 1904 (Gottlieb 1976). Cocaine is now widely used as an illicit addictive drug; global production between 1995 and 2002 was estimated to range from 640 to 950 tonnes used by an estimated 14 million people (United Nations 2002). The illicit growing of coca and its processing into cocaine has become a large and profitable industry that has had significant impacts on social and economic order in a number of producer as well as in consumer nations.

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 1,586 tonnes, of which about 160 tonnes were produced in South America (United Nations 2002). It is estimated that, globally, about 15 million people use opiates and that about 10 million of these use heroin (United Nations 2002). Like 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 medicinal drug when introduced to other parts of the world. While morphine is still used for medicinal purposes, heroin use is largely illegal and its production and distribution has significant socio-economic impacts in producer and consumer nations.

1.2 IMPACTS OF ILLICIT DRUG PRODUCTION IN COLOMBIA

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

Although the focus of this study is on the coca and poppy eradication program, it is important to recognize that the actual production of coca and poppy as well as the processing and production of cocaine and heroin involves 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 such as 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. As production is illegal, it normally takes place in remote locations. As a result, the clearing of land is done 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 (Table 1). 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 (Direccion Nacional de Estupefacientes 2002). Although some of these agrochemicals are highly toxic to mammals and may have significant environmental impacts, accurate information on the amounts used, their frequency of use, and the conditions of their use is not available. Because of this, it is 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 2004a, 2005).

Table 1. Pesticides used in the production of coca

Active ingredient	Toxicological classification[a]	Estimated % of use	Chemical class
Paraquat	II	61.3	Bipyrilidinium herbicide
Glyphosate	IV	19.1	Phosphate herbicide
2,4-D	I	9.7	Phenoxy herbicide
Atrazine	III	4.8	Triazine herbicide
Diuron	III	2.6	Urea herbicide
Carbaryl	II	NA	Carbamate insecticide
Carbendazim	III	NA	Benzimidazole carbamate fungicide
Carbofuran	I	NA	Carbamate insecticide
Chlorpyrifos	II	NA	Organophosphorus insecticide
Copper oxychloride	III	NA	Metal fungicide
Cypermethrin	II	NA	Pyrethroid insecticide
Diazinon	III	NA	Organophosphorus insecticide
Endosulfan	I	NA	Organochlorine insecticide
Lambda cyhalothrin	III	NA	Pyrethroid insecticide
Malathion	III	NA	Organophosphorus insecticide
Mancozeb	III	NA	Carbamate fungicide
Methamidophos	I	NA	Organophosphorus insecticide
Methomyl	I	NA	Carbamate insecticide
Methyl parathion	I	NA	Organophosphorus insecticide
Monocrotophos	I	NA	Organophosphorus insecticide
Prophenophos	II	NA	Organophosphorus insecticide

[a] As classified by the Instituto Colombiano Agropecuaria (ICA) as follows: I (very toxic), II (toxic), III (slightly toxic). Data from (Direccion Nacional de Estupefacientes 2002)

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 (Table 2). 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 health and the ecosystem. The toxicological and environmental properties of these substances are summarized in a separate Tier-1 Hazard Assessment Report (CICAD/OAS 2004a). Some of these substances have potentially large environmental and human health hazards and a subset of these are dealt with in more detail in Tier-2 Hazard Assessment Report (CICAD/OAS 2005).

Table 2. Identity and amounts of substances seized in Colombia as a result of counter-drug operations[a]

Year	1999	2000	2001	2002
Solid substances (units in Kg)				
Activated charcoal	36,681	49,323	84,141	93,057
Ammonium chloride	480	7	450	350
Ammonium nitrate	-	-	2,390	9,350
Ammonium sulfate	-	-	-	900
Calcium carbonate	500	150	255	1,570
Calcium chloride	7,371	33,073	56,985	146,040
Cement, grey	142,818	197,646	502,857	1,053,372
Cement, white	-	-	-	18,700
Lime	24,807	49,783	155,507	220,259
Potassium chloride	2,290	4,766	1,456	34,750
Potassium hydroxide	375	1,425	-	4,700
Potassium nitrate	2	-	2,150	2,390
Potassium permanganate (sum)	71,284	171,798	51,641	80,639
Sodium bicarbonate	52	4,827	8,538	9,939
Sodium carbonate	531,095	248,136	59,521	128,571
Sodium chloride	28,154	17,046	31,594	35,161
Sodium hydroxide	73,776	69,100	111,540	122,619
Sodium hypochlorite	-	16	4,208	1,720
Sodium sulfate	5,755	970	1,852	8,667
Urea	62,685	37,995	226,394	360,237
Liquid substances (units in L)				
Butyl Acetate	23,732	469	13,089	11,908
Ethyl Acetate	97,723	76,156	23,289	15,336
Acetone	1,666,474	894,070	1,546,651	1,841,860
Hydrochloric Acid	144,804	62,303	126,884	140,650
Sulfuric Acid	303,732	200,404	241,903	277,538
Isopropyl Alcohol	59,379	6,938	16,408	19,330
Ammonia	131,104	154,180	102,512	431,485
Acetic Anhydride	9,938	284	10,855	1,045
Chloroform	465	1,457	1	273
Ethyl Ether	205,984	67,704	53,989	110,098
Gasoline	621,686	1,034,880	2,013,650	2,612,820
Hexane	35,963	4,497		16,991
Kerosene	127,316	90,855	159,818	210,408
Methyl ethyl ketone MEK	88,402	69,209	10,674	41,332
Methanol	269,027	14,107	2,961	3,512

Table 2. Identity and amounts of substances seized in Colombia as a result of counter-drug operations[a]

Year	1999	2000	2001	2002
Methyl isobutyl ketone MIBK	55,943			2,086
Thinner	226,657	78,156	100,829	203,459
Toluene	3,630	208	19	6,469
Acetic acid	11	14	208	212
Nitric acid	59	6	1	5,300
Isobutyl alcohol	170		3	1,136
Petroleum ether				35,579
Methylene chloride	416	4	45	4,182
Fuel oil	32,082	325,250	346,460	948,083
Solvent No 1	203,603	116,498	435,816	280,921
Solvent No 2	6,505	3,819	5,621	11,942

[a] These substances are mainly used in the refining of cocaine, opium, and heroin. It is estimated that only 20% of the total amounts used are seized. Therefore, total use may be as much as 5-times greater than indicated in the table. Data from (Direccion Nacional de Estupefacientes 2002)

1.3 THE PROGRAM TO CONTROL ILLICIT DRUG PRODUCTION AND DISTRIBUTION IN COLOMBIA

The growing of coca and poppy and the distribution of cocaine and opium/heroin in Columbia has 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 Direccion 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 (Direccion Nacional de Estupefacientes 2002).

The aerial eradication program in Colombia is the responsibility of the Antinarcotics Directorate of the Colombian National Police (DIRAN-CNP), supported by data gathering from other nations such those in North America and Europe. The DIRAN conducts regular flights with aircraft that spray coca and opium poppy crops with 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. For example, at this time, certain existing or future alternative development projects or national parks may not be sprayed as a matter of policy.

Several concerns have been raised about the use of glyphosate and adjuvants in the eradication 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 the computer system.

The objectives of this assessment and report are to provide a science- and data-based study of the eradication program with a key focus on the environment and human health, to collect data for use in the assessment, to address specific concerns that have been raised, and to make the results known to the public and the scientific community. 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.

2 PROBLEM FORMULATION

The problem formulation is a key step in the process of the risk assessment 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 (Figure 2). Although the identity of the substances is known, 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 2004a, 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 estimated.

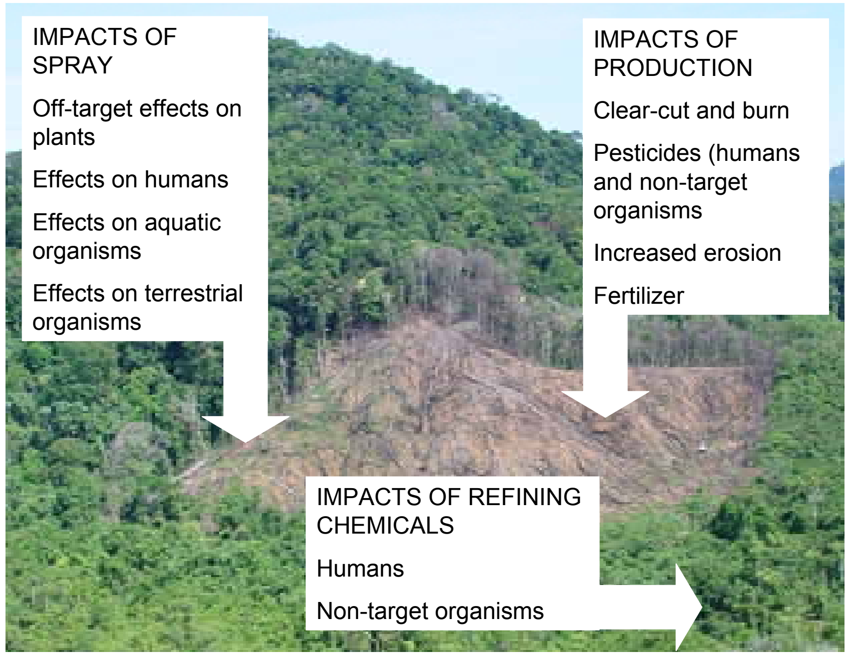


Figure 9 Diagrammatic representation of potential impacts of coca production, refining, and spraying.

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 a defined by eradication spraying and crops production patterns, must be used.

2.1 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.

2.1.1 Glyphosate

Glyphosate 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 probably greater since the introduction of glyphosate-tolerant crops (Wolfenbarger and Phifer 2000). Other agricultural uses for glyphosate-based products include its use by farmers as a routine step in pre-plant field preparation. Non-agricultural 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 in the literature (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.

2.1.1.1 Structure and chemical properties

The chemical name of glyphosate (acid) is N-(phosphonomethyl) glycine (MW = 167.09) and that of the most common technical form, the isopropylamine salt (IPA) is N-(phosphonomethyl) glycine isopropylamine salt (MW = 226.16). The Chemical Abstracts Registry (CAS) number of the acid is 114370-14-8 and for the IPA salt is 1071-83-6. The chemistry of glyphosate is important in determining its fate in the environment. Glyphosate (Figure 3) is a weak organic acid comprising a glycine moiety and a phosphonomethyl moiety. Chemically and physically, glyphosate closely resembles naturally occurring substances and it is not chemically reactive, 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).

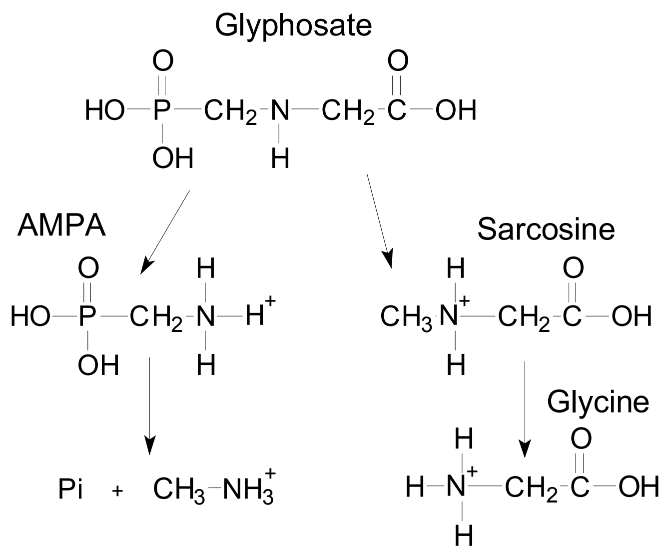


Figure 10 The structure of glyphosate and its major metabolic and breakdown products. From (Liu et al. 1991)

Glyphosate is readily ionized and, as the anion, will be strongly adsorbed to organic matter in soils of normal pH (Figure 4). It thus has low mobility in soils and is rapidly removed from water by adsorption to sediments and suspended particulate matter.

2.1.1.2 *Mechanism of action of glyphosate*

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). 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 non-toxic to animals but is an effective herbicide in plants.

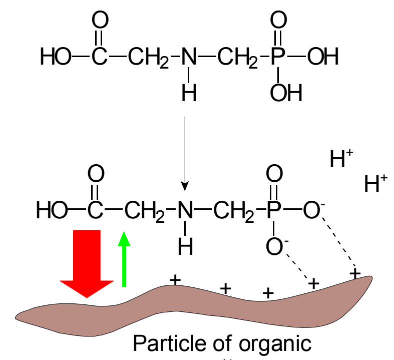


Figure 11 Binding of glyphosate to soil particles

2.1.1.3 *Global and local registration and use*

Glyphosate has been registered since 1971 and is currently widely used as a broad-spectrum, non-selective, post-emergence 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 non-selective herbicides that only affect the above-ground parts of the plant. Applied to soil, glyphosate shows low activity because the strong binding to soil organic matter makes the substance biologically unavailable to 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 3 and represents a relatively small fraction of the total use in Colombia.

Table 3. Use glyphosate in eradication spraying in Colombia 2000 to 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 (Direccion Nacional de Estupefacientes 2002, Policia Nacional Direccion Antinarcoticos 2005). [c] Estimated from total used in 2001 but likely less than this value.

2.1.1.4 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 below.

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, Figure 3), 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 great 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 (Figure 3) 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 (Figure 3). None of these products are considered herbicidal and they 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 light 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 (DT50) of 8-9 days under temperate forestry conditions (Feng and Thompson 1990). Similar rapid dissipation from fruits and lichen has also been observed (Stiltanen et al. 1981).

Dissipation under tropical conditions such as in Colombia 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, Central America, most of Africa between the Sahara and Kalahari deserts, India, inland Indochina, and portions of Northern Australia share similar tropical conditions and some of those countries 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 the pesticides in the tropics, they even concluded a greater rate of degradation under tropical conditions. The authors stated:

> *“Since soil microbial activities are strongly modulated by temperature, pesticide degradation would be expected to be greater in tropical soils, which experience higher year-round temperatures, than in temperate soils. This explanation would be consistent with observations of the elevated rates of soil organic matter turnover that characterize udic and ustic (rainy season) tropical environments. The few available studies which have directly compared pesticide fate in temperate and tropical soils held under identical conditions (i.e., laboratory) reveal no significant differences in either the kinetics or pathway of degradation. It appears that there are no inherent differences in pesticide fate due to soil properties uniquely possessed by tropical soils. Tropical soils themselves defy easy categorization, and their properties are as varied in nature as those from temperate zones. Pesticides appear to dissipate significantly more rapidly from soil under tropical conditions than under temperate conditions. The most prominent mechanisms for this acceleration in pesticide dissipation appear to be related to the effect of tropical climates, and would include increased volatility and enhanced chemical and microbial degradation rates on an annualized basis.*

2.1.2 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, ranging 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 non-target effects need to be minimized. In the control program in Colombia, an adjuvant, Cosmo-Flux®, is added at the time of spraying.

The relatively great water solubility and the ionic nature of glyphosate retard penetration through plant cuticular waxes (Figure 5). For this reason, glyphosate is commonly formulated with surfactants which 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).

2.1.2.1 Surfactants in the glyphosate formulation

The glyphosate formulation as used in eradication spraying in Colombia contains several formulants which are common to the commercial product as used in agricultural.

2.1.2.2 Cosmoflux 411F

As mentioned above, an adjuvant, Cosmo-Flux®, is added to the glyphosate at the time of spraying. Cosmo-Flux® is an agricultural adjuvant containing non-ionic surfactants (a mixture of linear and aryl polyethoxylates – 17% w/v) and isoparaffins (83% v/v) (Cosmoagro 2004). Adjuvants such as these are commonly added to pesticide formulations to improve efficacy through several mechanisms (Reeves 1992, Tadros 1994).

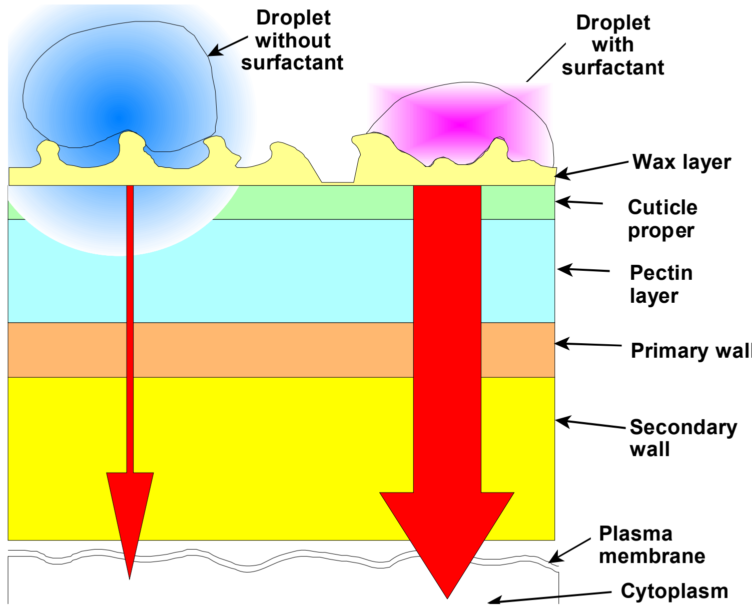


Figure 12 Penetration of an herbicide such as glyphosate through plant cuticular waxes in the absence (left) and presence of surfactants (right).

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 water insoluble wax cuticle, thus increasing the penetration of herbicide active ingredient.

Base oils, such as the isoparaffins in Cosmo-Flux®, are another class of adjuvants used in pesticide 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).

2.1.3 Coca and poppy control programs

As discussed briefly above, the coca and poppy control programs make use of several procedures to identify, locate, map coca and poppy fields. The initial step in this process is the use of satellite images to locate the coca and poppy 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 coca and poppy production. Further visual observations are made using overflights with observers and/or photographs from a low-altitude aerial-photography plane, such as a Cessna Caravan, to verify the presence of the coca and poppy fields. The camera used for this purpose is multi spectral high-resolution. Maps are generated in a Geographic Information System (GIS) and are used to produce updated co-ordinates for the spray pilots as well as information for downloading into the aircraft navigation systems

(Figures 6 and 7). The field operation offices for the control program have computers and a satellite uplink for data transfer. The spray-planes, such as AT 65s, AT 802s, or OV 10s, are equipped with high resolution tracking equipment and Del Norte positional data recorders that display position, provide directional guidance, and store positional data on data cards for later analysis. Thus the locations of the fields, the flight-paths of the spray-planes, and the areas where spray is released are known to within a resolution of several meters.

Since 1994, the coca and, more recently, poppy fields have been identified and sprayed during the eradication program. Total areas of identified fields, and area sprayed in Colombia are shown in Figure 8. With increasing areas sprayed, the total area planted to coca has generally decreased since 2000.

2.1.3.1 Receiving environment

Colombia is located between about 4ºS and 12°N of the equator. The country presents very 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, usual 2,200 m. The biodiversity hotspot for the tropical Andean region includes significant areas of Colombia (Figure 9). The tropical Andes biodiversity region is estimated to contain 15-17 percent of the world's plant life in only 0.8 percent of its area. It has a area of 1,258,000 square kilometers, and 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 the areas of coca production and regions of high biodiversity. Poppy is grown at greater altitude and this overlaps with the biodiversity hotspot; however, the total areas grown at this time are small (Figure 8). E xact 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 species of plants.

2.1.3.2 Method of application

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

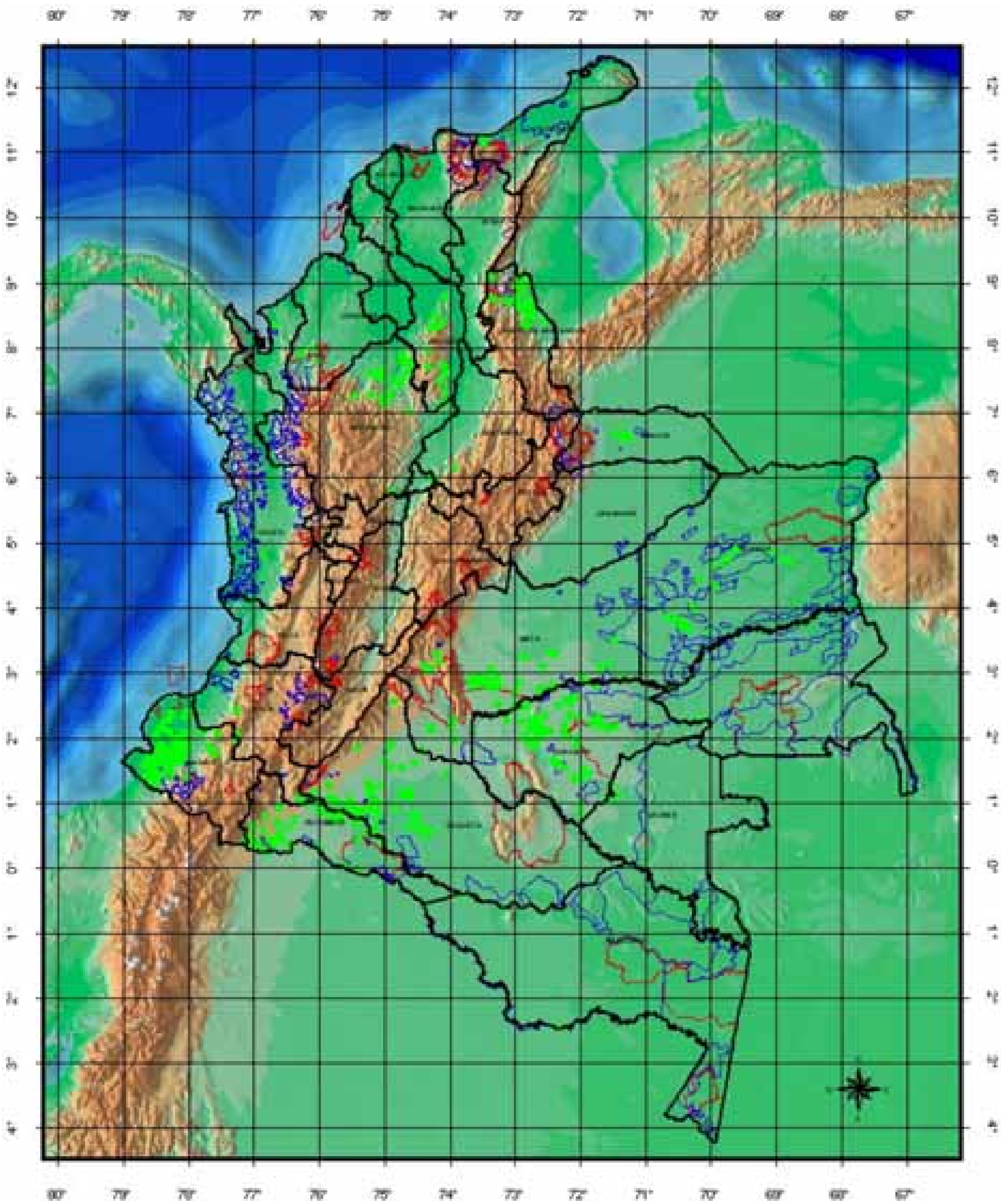


Figure 13 Map showing production of coca in Colombia in 2005. Bright green shows coca production. Blue boundaries indicate indigenous areas, red boundaries indicate national parks (Policia Nacional Direccion Antinarcoticos 2005).

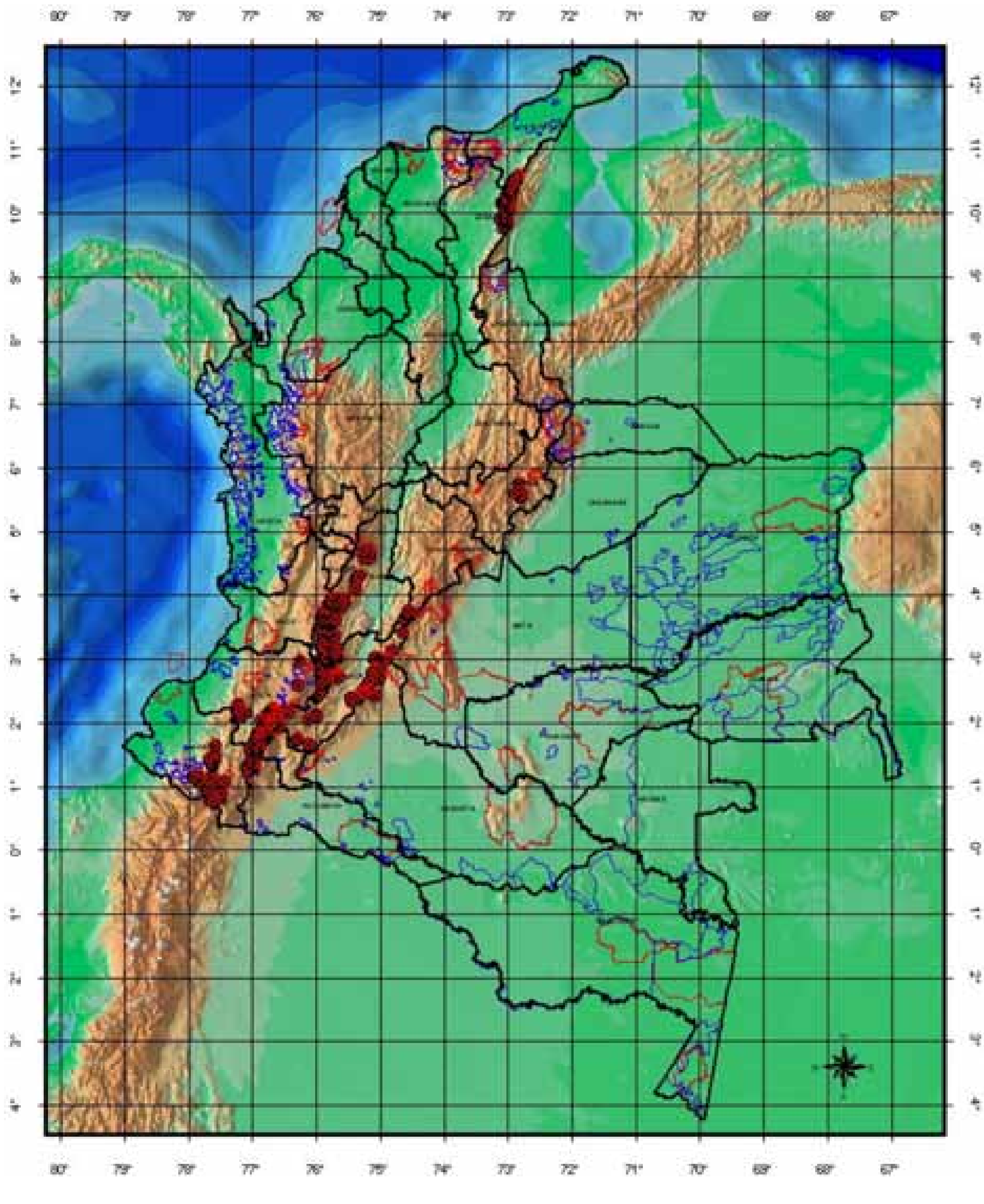


Figure 14 Map showing areas of poppy production in 2005. Bright red circles show poppy production. Blue boundaries indicate indigenous areas, red boundaries indicate national parks (Policia Nacional Direccion Antinarcoticos 2005).

The spray-planes are loaded in a special area of the tarmac at one of a number of bases throughout Colombia (Figure 10). 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. The glyphosate is transferred from 200-L plastic barrels to a larger plastic storage tank (Figure 10-A). 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 (Figure 10-B) into the aircraft using a Table of Mixing Proportions to ensure the correct ratio of amounts are loaded. Appropriate protective equipment is used by the mixer-loaders who are trained in the loading procedures (Figure 10-C).

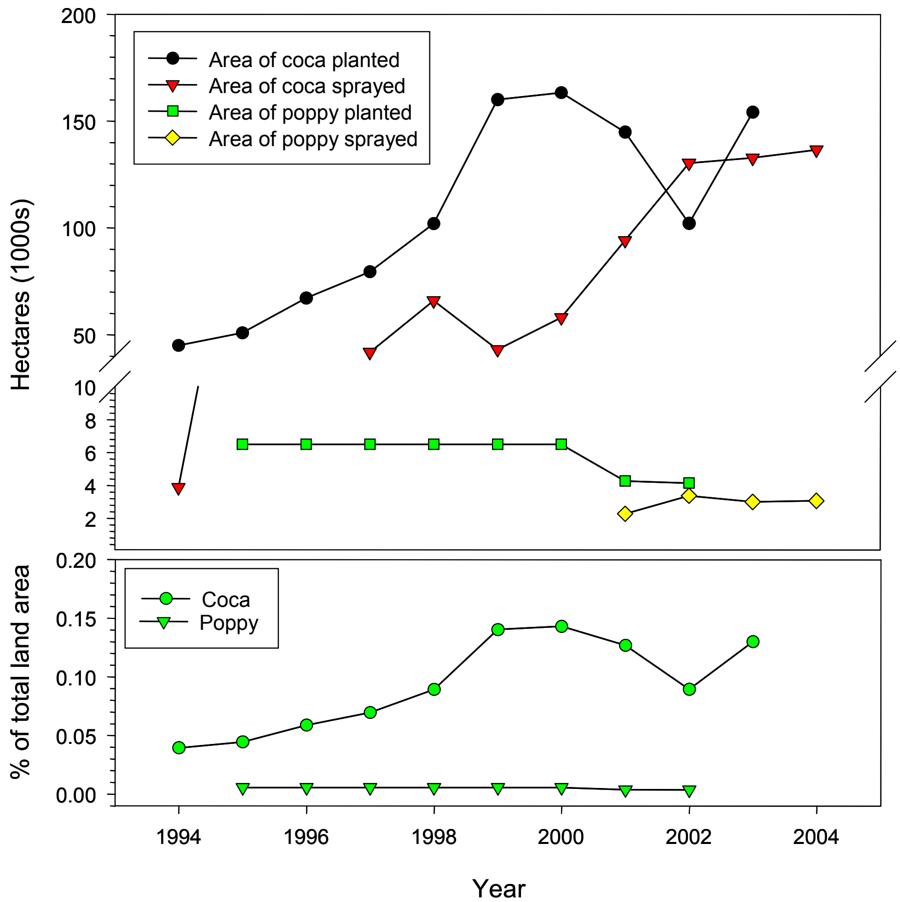


Figure 15 Areas planted with coca and poppy in Colombia from 1994 to 2002 as ha (above) and as a percent of the total land area of Colombia (below). From (Direccion Nacional de Estupefacientes 2002, Policia Nacional Direccion Antinarcoticos 2005)

The spray boom (Figure 10-D) on the aircraft is equipped with rain-drop nozzles (Figure 10-E). These nozzles produce droplets with a volume mean diameter (VMD) between 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 observing the readings of the spray pressure and the spray flow rate gauges (U.S. Department of State 2002).

Figure 16 Map showing the region of Colombia identified as part of the Andean Biodiversity Region. (From Centre for Biodiversity 2004).

The same nozzles are used for both coca and poppy applications but twice as many are used for the poppy applications and different boom pressures are used. As a result, coca and poppy applications are done at separate times. The currently-used application rates are shown in Table 4.

Table 4. Application rates of glyphosate and Cosmo-Flux® for control of coca and poppy

	Litres/ha		Kg/ha	
	Coca	**Poppy**	**Coca**	**Poppy**
Glyphosate	10.4	2.5	4.992	1.2
Cosmo-Flux®	0.24	0.51		

From (Direccion Nacional de Estupefacientes 2002)

Each spray operation (Figure 10-F and G), which may consist of 2 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 mid-afternoon to ensure that conditions are appropriate for application. If rain is imminent, visibility is poor, or the wind speed is in excess of 7.5 km/h (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. The 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 (Figure 10-H), 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 seen to be present.

After a spray operation, the flight path of the spray-planes and the areas sprayed is downloaded from the Del Norte system (Figure 10-I) and processed by GIS to show the spray patterns and calculate the areas spayed (Figure 10-J). This information is transmitted to the DIRAN where records of the spray operations are retained and used for compilation of annual reports and statistics (Direccion Nacional de Estupefacientes 2002).

2.1.3.3 Frequency of application

The frequency of application varies with the local conditions and the actions taken by the growers after the coca or poppy is sprayed. When coca is sprayed, some growers will 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 4-6 months. 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 to 12 months.

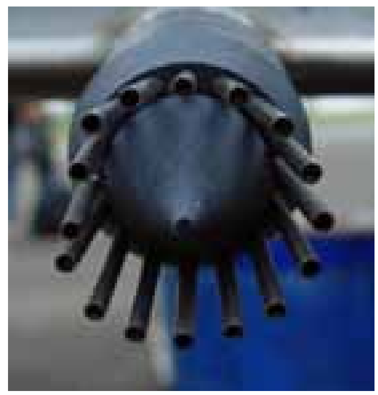
Being an annual, poppy is grown from seed. In the climatic conditions under which it is grown in Colombia, poppy fields would be harvested twice a year. If sprayed before reaching maturity and replanted immediately after spraying, they may be sprayed four times a year.

A) Mixing area for glyphosate and adjuvants

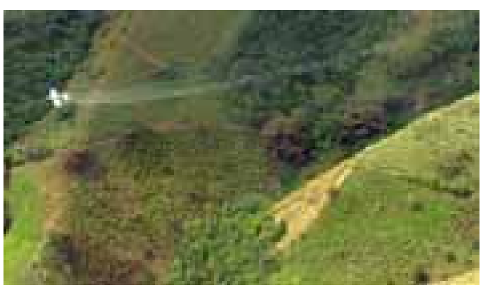
B) Mixer for glyphosate and adjuvant

C) Mixer-loader

D) Spray boom



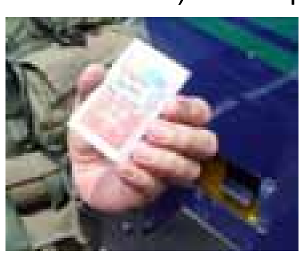
E) Nozzle



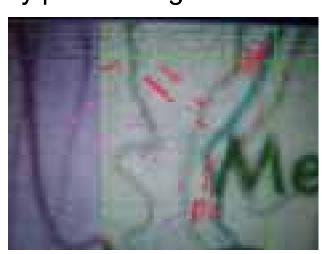
F) AT-65 spray plane

G) OV10 Spray plane being loaded

H) Del Norte GPS system



I) Positional data



J) Spray locations

Figure 17 Photographs of aspects of the spray operation (photographs K R Solomon).

2.1.3.4 Exposure pathways in soil, air, water, and other media

In terms of the application, there are several pathways through which the glyphosate and adjuvants may come into contact with the environment (Figure 11).

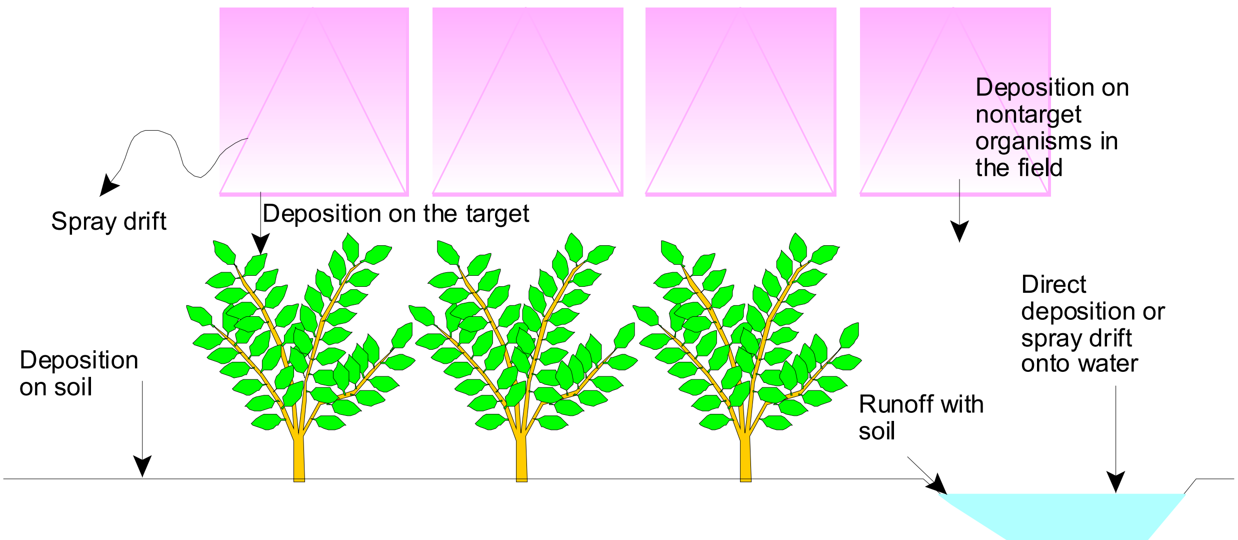


Figure 18 Diagram showing exposure routes for various environmental compartments when glyphosate is used for the control of illicit crops.

Deposition on the target crop (field) is the desired outcome of the operation; however, from the 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 the target field 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 the application. Depending on the depth of the water, turbulence, flow, and suspended particles, this would result in exposures of aquatic organisms to both glyphosate and any adjuvants present in the spray mixture. Organisms present in the field during spraying would be exposed to the spray droplets and would receive a theoretical dose, depending on surface area exposed and body mass. Exposures that may occur via these routes are discussed in Section 3.1.4.

2.1.3.5 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 non-target area on one or both sides of the target field. The second type of off-target deposition that may occur 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 the efficacy of the spray program (Helling 2003). Based on site-visits to 86 fields sprayed in 2002, and on 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 to the total area of coca sprayed (Figure 8) and calculating upper and lower intervals, the areas potentially affected are small when compared to the total area of Colombia (Table 5).

Table 5. Estimates of areas affected by off-target deposition of glyphosate in the spraying of coca in Colombia

Year	Ha sprayed	Area affected by off-target deposits (ha)		Upper interval as a % 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

While the areas affected by off-target are estimated to be small, this estimate is based on visual observations of a relatively small number of fields. These data were only available for coca, not poppy, however, the total areas planted to poppy at this time are not large, and similar off-target deposition would be proportionately smaller than that associated with coca production. This is thus a source of uncertainty in the assessment. It is not logistically possible to visually inspect all sprayed fields, however, the routine monitoring of the areas planted to coca and poppy that is undertaken by satellite and low altitude imagery could be used to assess any off-target deposition which results 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.

2.2 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. Although this document is focused on the risks associated with the coca and poppy eradication program, it is recognized that the eradication program is not conducted in isolation. There are a number of other activities associated with the process that result in risks to human health and the environment. While data are not available to quantify all these risks, some of them may be estimated on the basis of other knowledge and expert judgment. This was done using an adaptation of a risk prioritization scheme that has been used in ecological risk assessment (Harwell et al. 1992).

2.2.1 Context of the risks

2.2.1.1 Human health risks

Risks of the cycle of coca and poppy production were estimated as discussed above and are shown in Figure 12. For the purposes of this ranking process, 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. The scores for the risks associated with the eradication program were omitted from the ranking in this diagram but are discussed below in the conclusions to the risk assessment.

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	?	?	?	?	**?**
Processing and refining	5	3	5	75	**27.8**

Figure 19 Potential human health impacts of the cycle of coca or poppy production Scores for eradication spraying are specifically omitted.

2.2.1.2 Ecological risks

A similar procedure to that described above was used for ranking ecological risks associated with the cycle of coca and poppy production (Figure 13). 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 when clearing a natural area. Intensity of effects in this case also included off-field effects such as on non-target 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 the 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 years 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 four years 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.

Coca or poppy field developed in a natural area

IMPACTS	INTENSITY SCORE	RECOVERY TIME (Y)	IMPACT SCORE	% IMPACT
Clear cutting and burning	5	60	300	**97.6**
Planting the coca or poppy	1	4	4	**1.3**
Fertilizer inputs	1	0.5	0.5	**0.2**
Pesticide inputs	2	0.5	1	**0.3**
Eradication spray	?	?	?	**?**
Processing and refining	2	1	2	**0.7**

Figure 20 Potential environmental impacts of the cycle of coca or poppy production. Scores for eradication spraying are specifically omitted.

2.2.2 Conceptual model

For the purposes of the risk assessment of the use of glyphosate and adjuvants in the eradication of poppy and coca, the conceptual model applied was that normally applied to the agricultural application of pesticides where hazard and risk and directly related to the toxicity and exposure to the pesticide. 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 application of the sprays, exposure from this source is acute and resulting risks were compared to acute toxicity data. Toxicity data for the active ingredient, glyphosate, were obtained from the literature and from the results of acute laboratory-animal tests conducted with the mixture of glyphosate and Cosmo-Flux® as used in the spray program. It is recognized that glyphosate used in the eradication program may contribute to exposures via the food chain and drinking water; these were estimated and compared to toxicity data and exposure guidelines based on chronic toxicity for glyphosate. In addition, specific human health responses were assessed in epidemiological studies conducted specifically to address this issue in Colombia.

In assessing ecological risks, a similar agriculture-based approach was used. Similar to the above, 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 frequency of application in the eradication program (long periods between applications), ecological exposures resulting from the eradication spray operations were 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 below and the remainder of the document is focused on tests of these hypotheses.

2.2.3 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. Again, following the scientific method, 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 or chronic adverse outcomes on non-target organisms exposed via a number of routes

3 EXPOSURE CHARACTERIZATION

Exposure characterization is one of the key components to 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 pesticide containers, eating and drinking during pesticide application, and using inadequate protective clothing (Hurtig et al. 2003). However, agricultural uses such as these are quite different from the aerial applications of glyphosate for eradication of coca and poppy in Colombia. In the following sections, the potential for exposures in humans and the environment to glyphosate as used in the eradication program of humans is discussed and characterized.

3.1.1 Human exposure groups

In the case of human exposures to pesticides in the agricultural setting there are usually two groups that are considered – applicators and bystanders. The group that experiences the greatest probability of exposure is the applicator group, which, in this case, includes the mixer-loaders, the spray-plane pilots, and the technicians who work on and service the aircraft. The second group is the made up of 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 to the herbicide through the consumption of food items that have been sprayed, or drinking water that has been contaminated.

3.1.2 Applicator exposure

Risk to applicators was not a specific target of this assessment; however, exposure can be characterized for this group. Based on observations of the spray operations in several locations in Colombia, a number of measures are taken to reduce the potential for exposure of applicators (Table 6).

Table 6. Protective measures used to reduce exposure of applicators to glyphosate and formulants as used in poppy and coca eradication programs in Colombia.

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 in reduced.
Protective equipment worn.	Long pants, long sleeves, full rubber apron, rubber gloves, cloth hat or cap, particulate air filter and	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.

Table 6. Protective measures used to reduce exposure of applicators to glyphosate and formulants as used in poppy and coca eradication programs in Colombia.

Applicator subgroup	Mixer-loader	Spray pilot	Aircraft technician
	dark glasses, leather military-style boots.		
Equipment used to remove contamination, should it occur.	Eye-wash station at all locations, clean water for washing hand 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. From several studies, peak estimated exposure in applicators from all routes was 0.056 mg/kg body weight. The estimate of chronic exposure from all routes was 0.0085 mg/kg/day based on an 8 hour day and a 5 day 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 (Acquavella et al. 2004). In the spray program in Colombia, mixing and loading is done by one or two individuals who wear appropriate protective equipment. Pilots have limited opportunity for exposure and, as has been observed in other studies (Frank et al. 1985), will likely experience less exposure.

Exposures of mixer-loaders under the conditions of use 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 an agricultural applicator.

While most of the protective clothing worn by the 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 would offer 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.

3.1.3 Bystander exposure

Bystanders are the second group that can be exposed to glyphosate during application. Bystanders can be classified into several classes, depending on their route of exposure. These are discussed in the following sections.

3.1.3.1 Bystanders directly over-sprayed

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 solution to the body. There are several scenarios that could occur (Figure 14 and Table 7).

The most likely scenario is the partially clothed human with a cross-sectional area of 0.25 m² exposed to the spray (bold text in Table 7). 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 body weight.

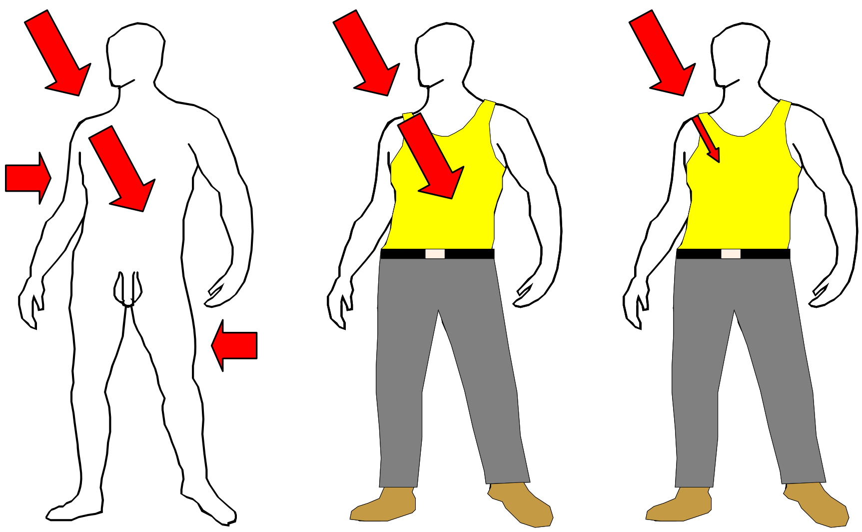


Figure 21 Illustration of human exposure scenarios

Table 7. Estimates of human exposure to glyphosate during a spray application

Scenario	Exposure in mg/kg body weight	
	Coca sprayed at 4.992 kg/ha	**Poppy sprayed at 1.2 kg/ha**
Naked human, total coverage of body, and complete penetration through skin.	14.2	3.4
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: (human weighs 70 kg and has a body surface area of 2 m^2

Bystander exposure to glyphosate was estimated as 0.0044 mg/kg/day for a child, 1-6 years 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 who were 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 urinary monitoring. The maximum systemic dose estimates for spouses and children were 0.00004 mg/kg and 0.0008 mg/kg, respectively (Acquavella et al. 2004). If bystanders are not directly sprayed nor reenter the field immediately after spraying, their exposures will likely be within a factor of 10 of farm bystanders. All of these measured exposures are considerably less than those estimated in Table 7. The values in Table 7 were thus considered to be reasonable worst-case values.

3.1.3.2 Re-entry

If a person was to reenter the sprayed field immediately after spraying and come into close contact with the treated foliage, such as when attempting to pick leaves from spayed 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 7. The potential for re-entry exposure has been summarized by Williams et al. (2000). Re-entry exposures decreased with time after application and, on day-7 after application, were 3% of those estimated for day 1. Re-entry into areas of tall weeds (1.5 m) resulted in 10-fold greater exposures than in areas of short grass. Based on measurements in farm workers, estimates of re-entry exposure to glyphosate in adults ranged from 0.0000039 to 0.0026 mg/kg/h of reentry time. Maximum re-entry exposure for a 1-6 year-old child was estimated at 0.026 mg/kg for a 5 hour contact period. As these estimates are based on a spray application rate of 1 kg/ha, re-entry exposures under Colombian conditions are estimated to be somewhat greater (Table 8). These numbers are also greater than the direct overspray as the people involved may have repeated exposures if they reenter a field immediately after spraying.

Table 8. Estimates of human exposure to glyphosate during re-entry to treated fields

Scenario	**Exposure in mg/kg body weight**	
	Coca sprayed at 4.992 kg/ha	**Poppy sprayed at 1.2 kg/ha**
Maximum re-entry exposure estimated for an adult human with a 10 hour day.	0.013	0.003
Maximum re-entry exposure estimated for a 1-6 year-old child with a 10 hour day.	0.259	0.062

3.1.3.3 Inhalation

Because the vapor pressure of glyphosate (isopropylamonium) is small (2.1 x 10^{-3} mPa at 25°C) and it also has a small Henry's Law Constant (4.6 x 10^{-10} Pa m^3 mol^{-1}) (BCPC 2002-2003), 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 of about 1000 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 h) resulting from inhalation of spray droplets by applicators was 0.0062 mg/kg (Williams et al. 2000), a value that is judged to be applicable as a maximum exposure for bystanders to eradication spraying in Colombia.

3.1.3.4 Dietary and drinking water

As shown in Table 9, dietary and drinking water exposures to glyphosate have been estimated to be relatively small under conditions of use in N. America (Williams et al. 2000).

Table 9. Worst-case daily human exposure estimates for glyphosate (mg/kg/day)

Sources	**Female adult**		**Female child (1-6 years)**	
	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/day.

Based on a study of 51 streams in nine Midwestern US States, the U.S. Geological Survey (USGS) reported the presence of glyphosate and a number of other herbicides in surface waters (Scribner et al. 2003). Of a total of 154 water samples collected during 2002, glyphosate was detected in 36 percent of the samples, and its degradation product, aminomethylphosphonic acid (AMPA) was detected in 69 percent of the samples. The highest measured concentration of glyphosate in any sample was 8.7 µg/L. The highest concentration of AMPA detected in the USGS study was 3.6 µg/L. Concentrations of glyphosate detected in surface waters in Colombia (see below) were, for the most part, less than 25 µg/L, the method detection limit. Exposures from drinking of untreated surface waters in areas where eradication spraying takes place are judged to be small and infrequent.

3.1.4 Environmental exposures

3.1.4.1 Air

As discussed above, the presence of glyphosate in air is unlikely as 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 the detection of glyphosate, along with other pesticides, in rainwater in the European Union (EU) (Quaghebeur et al. 2004). During the period 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.

3.1.4.2 Water

If water is directly over-sprayed during a spray operation, contamination of surface waters will result. Some coca fields are located near to ponds and lakes and some are near to streams and rivers (Helling 2003). While surface waters are not deliberately sprayed by the pilots, some over-spray 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 the fields, estimates of exposure were made using worst-case assumptions (Table 10) based on water depth assumptions used by the US EPA (Urban and Cook 1986) and the EU (Riley et al. 1991).

Table 10. Estimates of concentrations of glyphosate in surface water after a spray application

Scenario	**Exposure in µg/L (glyphosate[a])**	
	Coca sprayed at 4.992 kg/ha (3.69 kg AE/ha)	**Poppy sprayed at 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 to allow comparison to exposures used in environmental toxicity testing. In both these exposures and in the toxicity testing 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 has been detected in surface waters (see above discussion on human exposures through drinking water) in a number of locations. Glyphosate residues have been reported in surface waters in Denmark as result of agricultural activities. These residues were observed as part of the Pesticide Leaching Assessment Program (PLAP), a project that was intended to study the leaching potential of pesticides to the groundwater (Kjaer et al. 2005, Kjaer et al. 2003). 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 prior to 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 the soil would facilitate transport to the tile drains.

In the samples from PLAP collected following glyphosate applications, there were no detections of glyphosate or its metabolite, AMPA, that exceeded 0.1 µg/L in any of the groundwater samples taken from the suction cells (1 and 2 m below ground surface), the vertical wells (about 1.5 – 5.5 m below ground surface), and the horizontal wells (about 3.5 m below ground 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 µg/L and 0.06 µg/L, respectively, at a second location. At a third location, glyphosate and AMPA were detected but average concentrations of both were below 0.1 µg/L. In other studies 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 recent study on fate of glyphosate in soils showed rapid dissipation with almost total dissipation one month 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 as would be expected.

Other authors have reported glyphosate residues in surface waters in Europe (Skark et al. 1998, Skark et al. 2004) the frequency of detection was not large. The authors of these papers 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 run-off 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 non-agricultural pesticide use contributed more than two-thirds of the whole observed pesticide load in the tributaries and at least one-third in the River Ruhr (Skark et al. 2004). Most of the non-agricultural pesticides were derived from run-off 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, either of glyphosate or its metabolite, AMPA (aminomethylphosphonic acid) (Arregui et al. 2004).

The USGS study on Midwestern US streams (Scribner et al. 2003), analyzed samples of water that were filtered through a 0.7 µm filter, thus the concentrations represent dissolved glyphosate and AMPA. Summary data from this study are shown in Table 11.

Table 11. Summary data on glyphosate concentration in Midwestern US streams

Herbicide	**Number of samples**	**Concentration in µg/L**	
		95th centile	**Maximum**
Pre-emergence runoff samples			
Glyphosate	51	0.58	1.0
AMPA	51	0.55	1.8
Post-emergence runoff samples			
Glyphosate	52	1.5	4.5
AMPA	52	0.94	2.0
Harvest-season runoff samples			
Glyphosate	51	0.45	8.7
AMPA	51	1.3	3.6

Data from (Scribner et al. 2003)

Although the concentrations of glyphosate detected in surface waters in other areas where glyphosate 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 concentrations of glyphosate, AMPA and other pesticides in surface waters.

The surface water monitoring study was conducted in five locations in Colombia representing areas where spraying of coca was planned to take place or where other agricultural activities were undertaken and were also close to where human health studies were being conducted. The sites were selected for safe access as well as ease of repeated sampling. These locations are summarized in Table 12 and further details as to temperatures, rainfall, and soil characteristics are provided in separate reports (PTG 2005a, b, c, d, e)

Table 12. Characteristics of sampling sites for glyphosate, AMPA and other pesticides in surface waters and sediments in regions of Colombia

Site name	Location	Altitude (m)	Major crop types	Known pesticide use
Valle del Cauca, Río Bolo	N 03º27.642' W 076º19.860'	1002	Sugar cane	Glyphosate and other pesticides
Boyacá, Quebrada Paunera	N 05º40.369' W 074º00.986'	557	Coca	Manual eradication, no aerial spraying of glyphosate
Sierra Nevada, Quebrada La Otra	N 11º13.991' W 074º01.588'	407	Organic coffee	None
Putumayo, Río Mansoya	N 00º43.259' W 076º05.634	329	Coca	Aerial eradication spraying
Nariño, Rio Sabaletas	N 01º27.915' W 078º38.975'	15	Coca	Aerial eradication spraying

To characterize concentrations of glyphosate and AMPA in surface waters, samples were taken at weekly intervals for a period or 24 weeks (CICAD/OAS 2004c). Samples, in plastic bottles, were frozen and held at -17C until shipped to Canada for analysis using standard 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 bi-weekly 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 bi-monthly. Analytical quality control samples showed excellent recovery efficiency and precision of the analytical method with 98% recovery for glyphosate and 8.8% coefficient of variation (CV); 110% recovery efficiency for AMPA with 20% coefficient of variation. Blank field sample analyses show, on average, that no co-extractive interferences above the MDL for either glyphosate or AMPA at any of the sample sites. Field spike samples generally showed no significant degradation of glyphosate during sample handling and transport with overall average value of 90% of expected concentrations.

Results of these analyses are summarized in Table 13 (raw data are presented in Appendix 1). 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, concentrations of 30.1 and 25.5 μg/L, respectively, were found. These are sites where eradication spraying was not carried out and where the only use of glyphosate, if any, was in agriculture. These data suggest that 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.

Table 13. Concentrations of glyphosate (AE) and AMPA in samples of surface water collected in Colombia between October 2004 and March 2005

Site name	Total number of samples	Frequency of detection (n and %) for site	
		Glyphosate	AMPA
Valle del Cauca, Río Bolo	17	1 (5.9%)	0 (0%)
Boyacá, Quebrada Paunera	18	1 (5.5%)	0 (0%)
Sierra Nevada, Quebrada La Otra	18	0 (0%)	0 (0%)
Putumayo, Río Mansoya[a]	16	0 (0%)	0 (0%)
Nariño, Rio Sabaletas[a, b]	17	0 (0%)	0 (0%)

[a] Locations where eradication operations were planned.

[b] Location where eradication spraying was carried out during the sampling period.

To characterize concentrations of other pesticides in surface waters and sediments, samples of water were taken in glass bottles every two weeks for a period of 22 weeks (CICAD/OAS 2004b). Samples were held at 4ºC 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-week intervals as were sediment samples. Sediment blanks and spikes were taken once during the study period.

The results of the analyses for other pesticides are summarized in Table 14 (raw data are presented in Appendix 2A-G). Blanks showed no contamination of samples during storage and shipping. Spiked samples showed variable recovery, particularly for carbaryl. Several pesticides were detected in surface waters. This is not unexpected as pesticides are widely used in agriculture in Colombia and, based on similar 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 the 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.

Table 14. Concentrations of other pesticides in samples of surface water and sediments taken in Colombia between October 2004 and March 2005

Site name	**Number of samples**	**Frequency of detection in surface water**	
		Number	Pesticides detected
Valle del Cauca, Río Bolo	10	3	2,4-D
Boyacá, Quebrada Paunera	8	0	0
Sierra Nevada, Quebrada La Otra	9	0	0
Putumayo, Río Mansoya	9	0	0
Nariño, Rio Sabaletas	8	1	endosulfan I, endosulfan II, endosulfan sulfate
	Number of samples	**Frequency of detection in sediment**	
		Number	Pesticides detected
Valle del Cauca, Río Bolo	3	0	0
Boyacá, Quebrada Paunera	3	0	0
Sierra Nevada, Quebrada La Otra	3	0	0
Putumayo, Río Mansoya	3	0	0
Nariño, Rio Sabaletas	2	0	0

3.1.4.3 Soil

Concentrations of glyphosate and AMPA in soils can be estimated from the application rates used in the eradication program (Table 15) and 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.

Table 15. Estimates of glyphosate concentration in the top 25 mm of soil following a spray application		
Scenario	**Exposure in mg/kg**	
	Coca sprayed at 4.992 kg/ha	**Poppy sprayed at 1.2 kg/ha**
Direct deposition on bare soil with a density of 1.5 kg/L.	13.3	3.2
Deposition on soil with a density of 1.5 kg/L under a canopy of foliage with an assumed interception of 50%.	6.7	1.6

While 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 (Figure 15), from observation in other uses and other locations (Section 4.3.1), and from other reports (Helling 2003), this recolonization is rapid and there have been no adverse effects observed in terms of recolonization or replanting of the sprayed fields.

Figure 22 Photograph of coca plants near Caucasia, Colombia, replanted from cuttings in a field sprayed with glyphosate 56 days previously (Photo, K Solomon, 2004 06 09).

4 EFFECTS CHARACTERIZATION

4.1 GLYPHOSATE

The human-health and environmental effects of glyphosate have been extensively reviewed in the literature (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) [1]. 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).

4.1.1 Effects of glyphosate on mammals

4.1.1.1 Laboratory toxicity studies

The toxicity of glyphosate and the formulation Roundup® were reviewed recently (Williams et al. 2000). Glyphosate and its isopropylamine salt have low acute toxicity by the oral, dermal, and subcutaneous routes of exposure (Table 16).

Table 16. Acute toxicity of glyphosate in selected mammals

Species	Route	Compound administered[a]	LD50 (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	LC50=3.18 mg/L (4 hours)
	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

Data from (Smith and Oehme 1992).

[1] It should be noted that several publications on glyphosate have appeared in the literature which focus on the adverse effects of glyphosate. A pamphlet/brochure by Post (1999) was produced on behalf of an activist organization. The pamphlet was very brief and was not peer-reviewed. In addition, an article purporting to be a scientific review was published in 1998 (Cox , 1998) in the "Journal of Pesticide Reform". It should be noted that the Journal of Pesticide Reform does not publish original articles, is not peer-reviewed, is produced by an activist group, and that the editor is often the author of the articles. Because of this, these articles were not used in this report.

Toxicity was greatest by intraperitoneal administration. When rats and mice were given glyphosate orally or intraperitoneally, several stress symptoms, increased respiration, elevated rectal temperatures, and occasional asphyxial convulsions were noted. Median lethal doses of 4,704 mg/kg to the rat and 1,581 mg/kg to the mouse orally were significantly higher than 235 and 130 mg/kg, 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 calls recorded at the CNITV of France between 1991 and 1994. Only 31 cases were assessed as certain or highly probable and were linked with direct ingestion of 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 minutes and 2 hours. Recovery usually occurs over 1-2 days. 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 is noted occasionally. Rarely, jaundice, hepatic damage, and haematuria 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.

Some recent studies have examined effects of chronic feeding of glyphosate to Wistar rats. A study was performed to measure 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 which were exposed to glyphosate solutions 0.5% and 1% at a dose of 0.2 and 0.4 ml/ml water during 21 days of pregnancy. Glyphosate affects these enzymes in the studied organs of the pregnant rats and their fetuses (Daruich et al. 2001).

Feeding Glyphosate-Biocarbo® formulation at rates of 4.87 mg/kg every two days for 75 days resulted in the leakage of 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 nature of the formulants is unknown. In addition, the exposures extended over a long period of time and are inappropriate for assessing risks from acute and infrequent exposures such as may occur in eradication spraying.

The effect of glyphosate on several enzymes was studied *in vitro.* The enzymes were: serum acetylcholinesterase (AChE), lactate dehydrogenase (LDH), aspartate amino-transferase (AST), alanine aminotransferase (ALT, alkaline phosphatase (AP) and acid phosphatase (AcP). Results revealed that glyphosate inhibited all enzymes except AcP. IC50 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 aspartate amino-transferase was observed at a concentration of 54.2 mM, which is equivalent to a concentration of 9,056 mg/L, a concentration that would not occur in vivo. These results of the studies discussed above do not suggest that glyphosate would have effects at concentrations lower than those previously observed.

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

In a study on Charles River CD-1 rats, test animals were given oral gavage doses (direct intubation into the stomach) of 0, 300, 1000 and 3,500 mg/kg body weight (bw)/day of glyphosate from day 6-19 of gestation. Control animals received 0.5% methocel. No internal or skeletal anomalies were seen at 300 and 1000 mg/kg bw/day, although maternal toxicity was apparent at 3,500 mg/kg bw/day 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 were significantly increased at this dose level (Rodwell 1980a). Female Dutch belted rabbits were given oral gavage doses of 0, 75, 175, and 350 mg/kg bw/day glyphosate from day 6-27 of gestation. Control animals received 0.5% methocel. No internal or skeletal abnormalities were seen (Rodwell 1980b). In a study from Brazil, examination of pregnant Wistar rats dosed orally with Roundup® from day 6 to 15 of pregnancy with rates of 0, 500, 750, or 1000 mg/kg of glyphosate showed skeletal alteration in fetuses (15.4, 33.1, 42.0, and 57.3%, respectively). There was 50% mortality of dams at 1000 mg/kg only (Dallegrave et al. 2003). The doses used in this study 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/day was described for fetal effects and 300 mg/kg/day for maternal effects. Given the very large doses used in the Dallegrave et al. study (2003), their results are not surprising and do not change the assessment of teratogenic potential. The Rodwell studies discussed above also showed responses at concentrations greater than those reviewed in Williams et al. (2000) and do not change the assessment of teratogenic potential.

A number of recent studies have been carried out in tissue culture systems. One of these 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 did cause a reduction in steroidogenesis, but only for a period less than 24 hour during which there was recovery. In another study on tissue cultures, Lin and Garry reported results of bioassays carried out in cultures of the MCF-7 breast cancer cell (Lin and Garry 2000). The results presented by the authors indicated that, while some pesticides caused estrogen-like receptor mediated effects at high exposure concentrations, both glyphosate and the Roundup® formulation of glyphosate induced non-estrogen like proliferation, thereby supporting the view expressed by others (Williams et al. 2000) that neither glyphosate nor Roundup® are endocrine disruptors. The results of 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. They 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 on glyphosate 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 no 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 have appeared in the literature. Mice exposed to Roundup® at concentrations up to 1.05% in drinking water for 21 days showed no change in immune function (T-lymphocyte and macrophage-dependent antibody response) when, on day 21 of the herbicide 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 (1000 μM = 226,000 μg/L) (Nakashima et al. 2002). Results of both of these 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 minutes in 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 x 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 not formally used in assessment of pestcides by regulatory agencies.

The 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 hours and a terminal half-life of 79 to 337 hours in rats (Williams et al. 2000). Clearance from most tissues was rapid but it was cleared more slowly from the bone, possibly because of ionic binding to the calcium in the bones (Williams et al. 2000). Glyphosate is clearly not bioaccumulated and any absorbed dose is excreted in the urine relatively rapidly.

4.1.1.2 Cases of human poisoning

A number of anecdotal reports of human poisoning with glyphosate and formulations have been published in the literature. In some cases, these are reports of a single event and an observed response. In one such case 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 (Section 4.2.2).

In another case, a man accidentally sprayed himself with an unidentified formulation of glyphosate (Barbosa et al. 2001). He developed skin lesions 6 hours after the accident but these responded to routine treatment. However, one month 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 woman of 78 years old 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 strong acids. Lee et al. (2000) analyzed 131 suicide attempts in southern Taiwan. The most common symptoms were sore throat and nausea. 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 and mention that surfactant (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 pesticides (Krieger 2001).

It is well known that the older formulations of glyphosate that contained the surfactant POEA (MON 0818) 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 low. This observation is consistent with the minimal ocular and dermal effects observed with the formulation of glyphosate used in Colombia (Section 4.2.2).

4.1.1.3 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 made, and/or number of years of application. Recent studies have shown that these surrogates are susceptible to significant errors (Arbuckle et al. 2004), leading to the following quote from the authors of the paper:

> *"As the present analysis has shown, the consequences of this assumption could be a high false-positive rate in classification of exposure. The impact of this kind of error can be profound and has rarely been quantified. Until improvements are made in classifying pesticide exposure in epidemiologic studies, results on health effects will be subject to misclassification bias…."*

Similar conclusions have been put forward in other papers (Arbuckle et al. 2005, Harris et al. 2002, Solomon et al. 2005). A second possible source of error in these studies 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 Hardwell et al. (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 one related to a Hairy Cell Leukemia (HCL), a rare subtype of NHL. In the 1999 study, the authors employed a case control type of study design for their investigation. Case control studies can suffer from poor exposure histories and recall bias in that study subjects will be required to recall exposures to a putative agent which may have occurred decades prior to the onset of the disease under study. In some cases, study subjects may be deceased (in this study, 192 of the 442 study subjects were deceased) requiring exposure information to be provided by next of kin, thereby further eroding confidence in data related to exposure histories. The study reported their results in terms of odds ratio (OR). An OR of >1.0 implies a greater disease rate for exposed individuals than for the unexposed, while an OR <1.0 suggests a decreased rate of disease in the exposed population. The data for the study were based on small numbers; only four cases and three controls, or less than 1% of the overall study subjects, reported the use of glyphosate. Furthermore, the confidence interval (CI) reported by the authors for exposure to glyphosate was 0.4-13, implying a lack of statistical confidence. In their pooled analysis (Hardell et al. 2002), they reported a positive association with use of glyphosate (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 in 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 MN, 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 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 that had previously been reported by researchers in Canada and the US. 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 did express 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 relation 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 was observed for 6 of 13 pesticides categories, one 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. 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, while 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, we designed a preliminary study to gather human epidemiological data in several regions in Colombia. These regions were the same as those selected for the surface-water sampling (Table 12). The design and results of the study are summarized in the following section. A detailed report is given in a separate document (Sanin 2005).

4.1.2 Human health epidemiology study in Colombia

The question that this study addressed was: Is glyphosate exposure associated with adverse reproductive effects? The specific objective was thus to elucidate possible effects on reproductive health from exposure to glyphosate 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 (Table 17)

Table 17. Characteristics of the areas used in the epidemiology study

Site name	Focal crop	Known pesticide use
Valle del Cauca	Sugar cane	Glyphosate and other pesticides. Glyphosate applied by air.
Boyacá	Coca	Manual eradication, no aerial spraying of glyphosate. Use of other pesticides unknown.
Sierra Nevada	Organic coffee	No pesticide use and no coca known to be grown. Use of other pesticides unknown.
Putumayo	Coca	Aerial eradication spraying with lower intensity. Use of other pesticides unknown.
Nariño	Coca	Aerial eradication spraying with higher intensity. Use of other pesticides unknown.

The study protocol and questionnaire were approved through the Ethics Review Board of the Fundación Clínica Santa Fé de Bogotá, Colombia. All females of reproductive age in each area were informed about the objectives of the study and invited to participate if their first pregnancy (independent of the result of it) had occurred during the last 5 years, they had lived in the region at least for the same period, and they had not visited a physician for treatment of infertility nor used contraceptives during the year prior to getting pregnant. First pregnancies were the focus of the questionnaire. This reduced recall bias and other potential biases that are associated with subsequent pregnancies. Only one pregnancy was used to maintain outcome independence and minimize the effect of previous reproductive history.

Reproductive health was characterized through the following dependent variables (retrospectively) assessed by questionnaire:

Time to pregnancy (TTP): Number of months that it takes a couple to achieve a clinically detectable pregnancy without the use of contraceptives. A modified version of the key question from the questionnaire of Baird et al. (1991) was used to elicit TTP. Valid data on TTP can be derived retrospectively, with a recall time of 14 years or more (Joffe et al. 1995).

Fertility: Percentage of women who achieved pregnancy during the first year after intent.

The independent variable in the study was exposure to glyphosate for eradication of illicit crops. This was measured through use information from the region as indicated in Table 12. There were a number of possible confounders or independent predictors of the reproductive variables in study. These are listed below:

General Health and Nutrition Status	
Women and their partner	
Age	Complete years
Education	Highest grade achieved
Active smoking	Smoke or not; number of years number of cigarettes per day
Alcohol consumption	Number of drinks per month
Coffee consumption	Number of cups per day
Type of family	Nuclear or extended
Socioeconomic stratum	(Almost all all participants were stratum 1 – rural)
Only from Women:	
Body Mass Index	Weight (Kg) / (Height - m)
Reproductive history	Information on the father was also available

Techniques and procedures were as follows: In the five areas we started at the closest household to the location where water and sediment samples were taken from. Interviewers visited house by house to identify women who met inclusion criteria until the sample size (600 women in each zone) was completed. Those who met the inclusion criteria were informed about the project in a general way and were informed that there would no be reprisals for participation or non-participation and that the investigators guaranteed the privacy of the information collected. Each participant provided written informed consent.

Interviewers and supervisors were trained on the objectives of the project and the questionnaire for two days. All interviewers lived in the study area and were supervised by local epidemiologists who knew the study area and who were well known by the population. These local epidemiologists were supervised by PTG team. All the information collected was submitted to a quality control procedure. The data were captured in Microsoft Excel (Microsoft Corporation 2003) and processed with the STATA 7.0. (Stata Corporation, College Station, Texas) with macros developed by Dinno (2002). The modified version of the key question from the questionnaire of Baird et al. (1991) was used to elicit TTP was, “How many months were you having sexual intercourse before you became pregnant for the first time?" TTP was defined as duration in months, not divided by menstrual cycle duration in days, because women are more able to recall time in months than in cycles (Joffe 1997). For analysis purposes, if TTP was reported as zero months, the answer was interpreted as one month. Cutoff points for categorization of continuous variables were set as follows:

Age at time of interview - 25;

Age when started to try to get pregnant and age when first got pregnant - 20.

Of a total of 3005 women interviewed, 413 exclusions were made. These included: 233 women without TTP data and 21 with TTP values greater than 60 months and 159 women who consulted to physician about infertility. Hence, 2592 (86.3%) were included in the analyses reported here.

For each exposure and potential determinant variable, non-parametric ANOVAs of TTP were conducted. In the fecundability predictor models, censoring of TTP was introduced, in order to reduce the effect of other medical causes on TTP. If a woman took more than 12 months to conceive, a value of "null" for a separate censor variable was included with a value equal to 0 if TTP was 12 months or less and 1 if TTP was greater than 12 months.

Each month was classified according to the ecological exposure and determinant variables and an indicator variable was generated for every month giving information on whether the cycle under this exposure resulted in a pregnancy or not. Fecundability odds ratios (fOR) were calculated with 95% confidence intervals (95% CI) using a discrete time analogue of Cox's proportional hazard model (Baird et al. 1986, Curtis et al. 1999, Zhou and Weinberg 1999). This process generate a fOR for which values below unity indicate sub-fertility.

The initial saturated multivariate model included all variables significant on bivariate analysis ($p<0.10$) and variables of prime biological importance (age at time of trying to get pregnant). Variables were eliminated one by one according to the p values (>0.05) and effects of elimination on the coefficients of other variables in the model assessed. Several goodness of fit statistics for logistic regression were checked (Hosmer and Lemeshow 1989). The final model consisted of only those variables that contributed to the explanatory value of the model (coefficient of determination). Co-linearity was tested with VIF (Variance Inflation Factor). The assumption that the fOR was constant across time (Weinberg and Wilcox 1998) was tested graphically and by including an interaction term between cycle (time) and exposure or determinant variables in the final model. The latter were not significant, implying that the proportional assumption was not violated. Finally, to evaluate a possible selection bias based on willingness to participate, the analyses were repeated excluding the pregnancies occurring by the first month (Weinberg et al. 1994). No significant changes in the final model were observed.

The distribution of pregnancies in relation TTP (Figure 16) was different between the five regions. In previous work in Colombia (Idrovo et al. 2005), the percentage for first month was about 30% - low compared with data from developed countries. In this case, Valle del Cauca had very low initial percentage and Boyacá had high values for the first and twelfth months (Figure 1). The mean for 12 months in developed countries is 85-90%.

Participating women were generally young (mean and median age 21 years old) and had completed at least some secondary education. The vast majority had regular menstrual cycles (96.7%); a substantial proportion had irregular partner relationships. Most experienced their first pregnancy at young ages (73.6% at < 20 years). During the year before first pregnancy, most were free of illness (84%), had not had x-rays (95.4%), and did not smoke tobacco (95.1%). Alcohol and coffee consumption were 51.8% and 80.3% respectively. The majority of women were housekeepers at the time of first pregnancy.

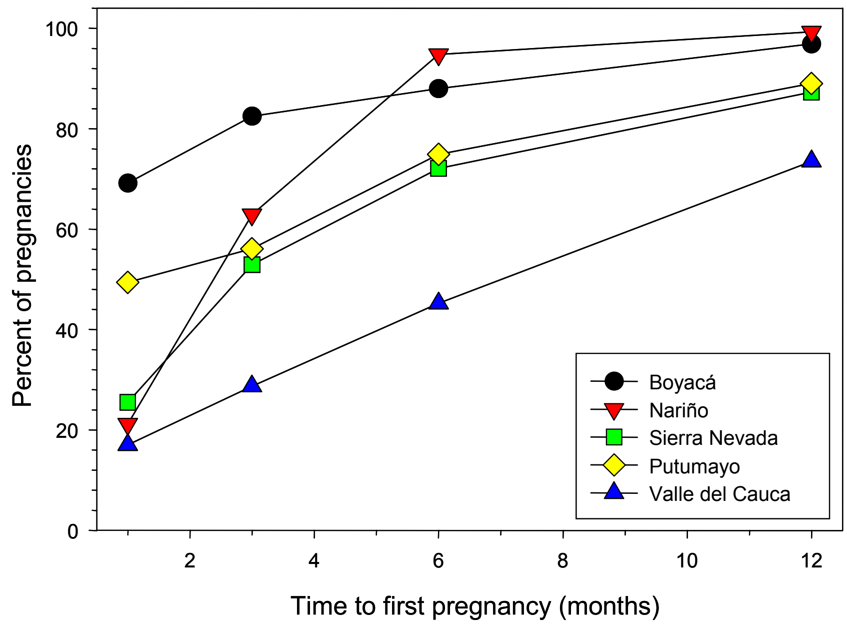


Figure 23 Summary of the results of the time to pregnancy study

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. Previously visit to 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. Maternal overweight was associated with a longer TTP. A tendency to longer TTP was observed among those engaged in some waged work and with higher education. Paternal unemployment or self work, were associated with longer TTP. No other paternal data were related with the TTP.

After adjustment of the model for region, several associations were identified (Table 18). Although non-significant in the adjusted model ($p< 0.1$), coffee intake and self perception about bad quality of water was associated with longer TTP and all sources of water presented greater risk of longer TTP when they were compared with pure water ("nacimiento"), except for a few cases which use carried water ("carro-tanque").

Table 18. Causes of fecundability adjusted [a] for the relationship between time to preganacy (TTP) and region [b] based on an alternative model.

Variable	fRMa [c]	SE [d]	95% CI [e]	*p*
Region [f]				
Nariño	0.56	0.048	0.47, 0.66	<0.01
Sierra Nevada	0.36	0.031	0.31, 0.43	<0.01
Putumayo	0.35	0.029	0.29, 0.41	<0.01
Valle del Cauca	0.15	0.014	0.13, 0.18	<0.01
Age at first preganacy > 20 years [g]	0.81	0.048	0.73, 0.91	<0.01
Irregular relationship [h]	0.76	0.041	0.68, 0.84	<0.01
Consumption of coffee [i]				
Medium (1-3 cups per day)	0.91	0.059	0.81, 1.04	0.15
High (4 and more cups per day)	0.84	0.083	0.69, 1.02	0.08
Perception of contamination of water [j]	0.91	0.51	0.81, 1.01	0.08

n = 2592 mothers 11,270 cycles.

[a] Proportional risk model of Cox, modified after Dinno (2002) Modelo de Riesgos proporcionales de Cox, modificado (Dinno, 2000). [b] Restricted to those mothers who did not consult a physician regarding problems in conceiving. [c] **fRMa** Adjusted cause of fecundability. [d] Standard Error. [e] 95% confidence interval. [f] Compared to Boyacá as reference. [g] Compared to ≤20 years as reference. [h] Compared to regular relationship as reference. [i] Compared to no consumption as reference. [j] Compared to no contamination as reference and based on self-perception and source of water normally consumed.

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 higher risk. The greatest risk was in the Valle del Cauca region. There was no association between TTP and use of herbicides in the eradication 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 since Sierra Nevada, where organic crops are grown, also showed a statistically significant difference from the reference location (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 in our study should be included in any future studies that may be conducted.

4.1.3 Effects of glyphosate in non-target 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 inhibits the shikimate pathway by blocking 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS). This reduces the synthesis of aromatic amino acids and causes accumulation of high concentrations of shikimic acid and its derivatives. Glyphosate translocates to active growing tissues, 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. 2002, Roberts et al. 1998). However, toxic effects of the compound on, for example, non-mammalian 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 non-temperate 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 related pesticides become more toxic at lower temperatures (Dyer et al. 1997); however the mechanisms here are well understood. Comparison of responses of tropical and temperate organisms to a number of pesticides other than DDT has shown that there are not significant differences in sensitivity (Maltby et al. 2005). With this in mind, we used the rich data set of toxicity values that has accumulated in the literature for glyphosate and its formulations.

4.1.3.1 Effects in non-target terrestrial animals

The potential environmental effects of glyphosate and Roundup® were extensively reviewed in 1999 (Giesy et al. 2000). Some additional papers have appeared since that time. Glyphosate is not considered directly toxic to terrestrial organisms.

Soil invertebrates. The effects of glyphosate 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 of 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. This amount of glyphosate is 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 x 12.6 cm or 159 cm^2. This being so, the application rate used by the authors 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 obviously seriously flawed and the results are not applicable to any use of glyphosate. This study has no relevance to the use of glyphosate for the control of illicit crops in Colombia.

Soil microorganisms. Glyphosate has little effect on soil microorganisms (Giesy et al. 2000). Since the symbiotic soil and root-associated microorganisms may be partially dependent on the plant for nutrients, the death or injury of the plant will result in effects on the organisms associated with it. Similarly, death of the plants would release organic matter and nutrients into the soil and this would affect soil microorganisms in a similar way to the application of compost or fertilizer. This effect was reported for glyphosate and its effects on grass (Tenuta and Beuchamp 1995). This 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 the 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 at concentrations of 0, 1, 10, 100, and 1000 mg/Kg 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 technical 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. 2002, Roberts et al. 1998).

Analysis of all lines of evidence for effects of glyphosate 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, different soils over the past 10 years and under various cropping systems, Motavalli et al. (2004) have concluded that so far no conclusive evidence shows that glyphosate has any 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 of use. Glyphosate binds strongly to soil particles and would not be available for uptake by these microorganisms, many of which are actually inside the tissues of the plants. 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 conditions of use.

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

After applying glyphosate at double the recommended application rates, no effects were observed in microarthropods in soil (Gomez and Sagardoy 1985). As weed species compositions and densities are directly affected by the glyphosate, indirect effects are more likely to occur. Jackson and Pitre (2004a) found that populations of adult *Cerotoma trifurcata*, adult *Spissistilus festinus*, larvae of *Plathypena scabra*, and the caterpillar of *Anticarsia gemmatalis* were unaffected by glyphosate but, populations of adult *Geocoris punctipes*, a Hompoteran insect predator, were decreased by the herbicide. The authors concluded that this effect was due to reduced weed densities after glyphosate treatment. Populations of green cloverworm (*Hypena scabra*) were evaluated on soybean glyphosate-resistant varieties, with and without exposure to glyphosate 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 United Kingdom, indirect effects of glyphosate were observed in the spider *Lepthyphantes tenuis.* These were a result of habitat alteration and were related to death of plants and decreasing height of vegetation. Glyphosate applications only had a within-season indirect habitat effect on *L. tenuis* as field margins sampled 16 months after an application of 360 g glyphosate/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 a 10-d post-treatment period. Exposure of *G. punctipes* eggs to glyphosate spray had no effect on egg hatch (Jackson and Pitre 2004b). Some reductions in numbers of this species 3 weeks after treatment probably reflect weed removal, i.e. habitat alteration (Jackson and Pitre 2004a).

Similarly, studies on populations of leaf litter invertebrates in areas of Australia where glyphosate was spayed at 1 to 1.4 kg/ha for the control of an invasive weed, showed no significant effects four months after spraying (Lindsay and French 2004). The authors pointed out that variability in treated and untreated areas was large and suggested that the nature of the vegetative community and its structure and the post-spray 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, and glyphosate mixed with Cosmo-Flux® are not acutely toxic to mammals via several routes of exposures (reviewed in this report). Although wild mammals have not been specifically tested with the mixture as used in Colombia, the 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 LD50 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 very unlikely.

Indirect effects on terrestrial wildlife have been reported to be associated with the use of glyphosate in agriculture and forestry uses. Alteration of habitat is more of an issue in semi-wild areas such as forests where herbicides may be used to control competing vegetation and allows 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 years (Kimball and Hunter 1990, Santillo et al. 1989a, Santillo et al. 1989b) and even the vegetation will recover in less than ten years (BC Ministry of Forests 2000, Boateng et al. 2000). Normally, in these uses, the actual areas treated with herbicides 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 to these animals (Giesy et al. 2000, World Health Organization International Program on Chemical Safety 1994).

Glyphosate is widely used for vegetation management, including in the restoration of native plant communities where exotic or invasive species are controlled, (e.g. 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, (e.g. Sullivan and Sullivan 2003). A review of management of northern US forests, including the use of herbicides including 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 has effects on bird communities in British Colombia, 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 areas treated with herbicide and may be particularly susceptible to the indirect effects of glyphosate application on plant removal (Easton and Martin 1998, Easton and Martin 2002).

Nevertheless, control of *Cirsium arvense* (Canada thistle) using wick application of glyphosate in wildfowl areas can enhance plant diversity that is of benefit to water birds (Krueger-Mangold et al. 2002). However, the broad spectrum activity of glyphosate means that accidental overspray of rare non-target 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 LD50 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 surfactants added to the mixture. 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). This was done in accordance with the testing methods and guidelines developed by the Organization for Economic Cooperation and Development (OECD) Method #214, “Honeybees, Acute Contact Toxicity Test” (OECD 1998a) and the United States Environmental Protection Agency (U.S. EPA) Office of Prevention, Pesticides and Toxic Substances (OPPTS) Ecological Effects Test Guideline 850.3020, “Honey Bee Acute Contact Toxicity” (USEPA 1996a). The results of this study showed that the mixture of glyphosate and Cosmo-Flux® 411F is acutely nontoxic via contact exposure to honey bees (i.e., did not cause mortality or stress effects in bees within 48-hours of treatment) at concentrations equal to or less than 56.8 mg AE/bee. These results are similar to those for glyphosate and formulations from the US EPA ECOTOX data base (USEPA 2001) and show that the formulated product as used in Colombia is not hazardous to bees and, by extrapolation, to other beneficial insects.

4.1.3.2 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 recent publications have 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 frogs was measured (Mann and Bidwell 1999). The authors reported the acute toxicity for adults of one species and tadpoles of four species of southwestern Australian frogs in 48-h static/renewal tests. The 48-h LC50 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/L glyphosate acid equivalent

[AE], while the 48-h LC50 values for Roundup® Herbicide tested against adult and newly metamorphosed *C. insignifera* ranged from 137,000-144,000 µg/L (49,400-51,800 µg/L AE). 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-h LC50 values ranging between 27,300 and 48,700 µg/L (9,000 and 16,100 µg/L AE). Roundup® Biactive (MON 77920) was practically nontoxic to tadpoles of the same four species producing 48-h LC50 values of 911,000 µg/L (328,000 µg/L AE) for *L. moorei* and >1,000,000 µg/L (>360,000 µg/L AE) 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 h, at concentrations between 503,000 and 684,000 µg/L (343,000 and 466,000 µg/L AE). The toxicity of technical-grade glyphosate acid (48-h LC50, 81,200 -121,000 µg/L) is likely to be due to acid intolerance. Slight differences in species sensitivity were evident, with *L. moorei* tadpoles showing greater sensitivity than tadpoles of the other four species. Adult and newly emergent metamorphs were less sensitive than tadpoles.

A series of studies on frogs were 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, LC50 values as low as 880 µg/L (as glyphosate) were reported for tadpoles of *Xenopus laevis*, *Bufo americanus*, *Rana clamitans*, *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/L), LC50 values could not be determined.

Field studies conducted on larvae of *Rana clamitans* and *Rana pipiens* with the Vision® formulation of glyphosate showed that, in the presence of natural factors such as sediment and environmentally relevant pH, the toxicity of the formulation was reduced as compared to laboratory observations (Wojtaszek et al. 2004). The authors reported 96-h median lethal concentration (LC50) values ranging from 2,700 to 11,500 µg/L (as glyphosate) (Wojtaszek et al. 2004). Although the authors 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 and this further reduces risks, a conclusion reached for this forestry use (Thompson et al. 2004) but which is equally relevant to the use of glyphosate in Colombia.

In another study on amphibians, the toxicity of a number of glyphosate formulations to frogs (*Rana clamitans, R. pipiens, R. sylvatica*, and *Bufo americanus*) was reported (Howe et al. 2004). The formulations included Roundup Original®, glyphosate technical, the POEA surfactan used in some glyphosate-based herbicides, and five newer glyphosate 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-h LC50 >17,900 µg/L). LC50 values for Roundup Original® in *R. clamitans, R. pipiens,* and *R. sylvatica* were 2,200, 2,900, and 5,100 µg/L (AE), respectively. These values were used in this risk assessment. Several other formulations of glyphosate were also tested in *R. clamitans* and these (Roundup Biactive®, Touchdown®, and Glyfos BIO®) were essentially non-toxic with LC50 values of >57,000 µg/L.

In a study carried out with several commercial pesticide formulations in leopard frogs (*Rana pipiens*), green frogs (*R. clamitans*), bullfrogs (*R. catesbeiana*), the American toad (*Bufo americanus*), and gray tree frogs (*Hyla versicolor*), effects of Roundup® and interactions with other pesticides were reported (Relyea 2004). The formulation of Roundup® used in this study contained the more toxic POEA surfactant. Survival and growth over a 16 day period were not significantly affected by the glyphosate formulation at 1,000 µg/L (glyphosate AE) but some species were affected at 2,000 µg/L. Some interactions were observed between the glyphosate formulation and other pesticides such as the insecticides diazinon, carbaryl, and malathion. A recent paper reported that a glyphosate formulation containing POEA was highly toxic to tadpoles of several species of frogs exposed under realistic conditions in small (1000-L) field microcosms (Relyea 2005). 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 a concentration of 3,800 µg/L (AE) of glyphosate formulation applied as a commercial formulation (unspecified) directly to the surface of the water. The rate of application was equivalent to 16 kg/ha, a value that is unrealistic and probably the result of an error in the methods. 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 be having adverse effects on frogs thus based on a flawed study design and is not supported by other data, much of which is discussed above.

Effects on other non-target aquatic organisms have also been recently reported in the literature. 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 the product Roundup®, which is formulated with the POEA surfactant. LC and EC50 values for technical glyphosate ranged from 5,890 to 415,000 µg/L. In tests conducted in the presence of sediment (Tsui and Chu 2004), these same authors showed that biological availability of glyphosate was significantly reduced by binding to sediment. The reduction in porewater concentration that resulted from the presence of sediments was proportional to the amount of organic carbon in the sediments.

Tests on the fish *Oreochromis niloticus* (Nile tilapia) exposed for 3 months 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 damages 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 sublethal concentrations had caused histopathological and biochemical alterations of the fish (Jiraungkoorskul et al. 2003). Because technical glyphosate was not tested and the contribution of the surfactants to this response cannot be judged.

In studies on the freshwater mussel *Utterbackia imbecillis*, a commercial formulation of Roundup® was reported to have low toxicity (24-h LC50 of 18,300 µg/L and a No Observed Effect Concentration (NOEC) of 10,040 µg/L – 7,442 µg/L AE) 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 ¼ the NOEC, ≈ 2,500 µg/L (1,850 AE).

Response of total free amino acids profiles of snails to glyphosate exposures has been studied (Tate et al. 2000). These authors showed that exposure of the aquatic snails (*Pseudosuccinae columella*) to technical glyphosate at nominal concentrations of 1000-10,000 µg/L lead to increased egg-laying and increased amino acid concentrations in the tissues. Technical glyphosate was not particularly toxic with a 24-LC50 of 98,900 µg/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* where growth rates and sexual and asexual reproduction were stimulated in the presence of glyphosate (formulated, but formulation unknown) at concentration of ≥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 the 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 the growth of the algae (Bengtsson et al. 2004). Stimulation of algal growth was suggested to be due to release of nitrogen and phosphorus from the metabolism of glyphosate by the *Daphnia*. Similar stimulation was also seen in the effects of glyphosate (Rodeo®, a formulation without any 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 glyphosate 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.

The effects of glyphosate on fish and other aquatic organisms are clearly related to the surfactant in the formulation rather than the glyphosate itself. Surfactants can disrupt cell membranes and this type of response would be expected. For this reason, the glyphosate formulation and the surfactant (Cosmo-Flux®-411) as used in Colombia for the eradication of coca and poppy were tested for toxicity to the aquatic organisms, algae, crustacea, and fish (Section 4.2.2). The protocols used are described below and results are summarized in Table 19.

Algal tests. The testing of a mixture of a commercial formulation of glyphosate and the surfactant Cosmo-Flux® 411F, was conducted to determine growth inhibition of the freshwater green alga, *Selenastrum capricornutum* Printz, according to the Organization for Economic Co-operation and Development (OECD) Method # 201, "Alga, Growth Inhibition Test" (OECD 1984b) and in general accordance with OPPTS Method 850.5400, "Algal Toxicity, Tiers I and II" (USEPA 1996b).

Water Flea. Tests were conducted to determine the acute toxicity of a commercial formulation of glyphosate and the surfactant Cosmo-Flux® 411F to *Daphnia magna* according to OECD Method #202, "*Daphnia sp*., Acute Immobilization Test and Reproduction Test" (OECD 1984a), however, the reproduction component of the test was not conducted.

Rainbow Trout and Fathead Minnow. Tests were conducted to determine the acute toxicity of a commercial formulation of glyphosate and the surfactant Cosmo-Flux® 411F to *Oncorhynchus mykiss* and *Pimephales promelas* according to OECD Method #203, "Fish, Acute Toxicity Test" (OECD 1992). In all of these tests, OECD Principles of GLP (OECD 1998b) were followed.

Table 19. Toxicity values obtained from toxicity tests conducted on a mixture of glyphosate and Cosmo-Flux®.

Test species	**Common name**	**96 hour LC/EC50 in µg/L (as glyphosate AE)**	**Reference**
Selenastrum	Algae	2,278-5,727[a]	(Stantec 2005b)
Daphnia magna	Water flea	4,240 (3,230-5,720)[b]	(Stantec 2005e)
Onchorynchus mykiss	Rainbow trout	1,850 (1,410-2,420)[b]	(Stantec 2005c)
Pimephales promelas	Fathead minnow	4,600 (1,810-1,173)[b]	(Stantec 2005d)

[a] Lowest and highest effect measures in the study

[b] LC/EC50 and 95% Confidence Interval

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 used in Colombia (Figure 17). 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). These data show that the combination of formulated glyphosate and Cosmo-Flux®, as used in Colombia, is more toxic to the aquatic organisms tested than formulations without the addition of surfactants and adjuvants. This is not altogether surprising. It has been shown that 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 is clearly the cause of the increased toxicity of the mixture.

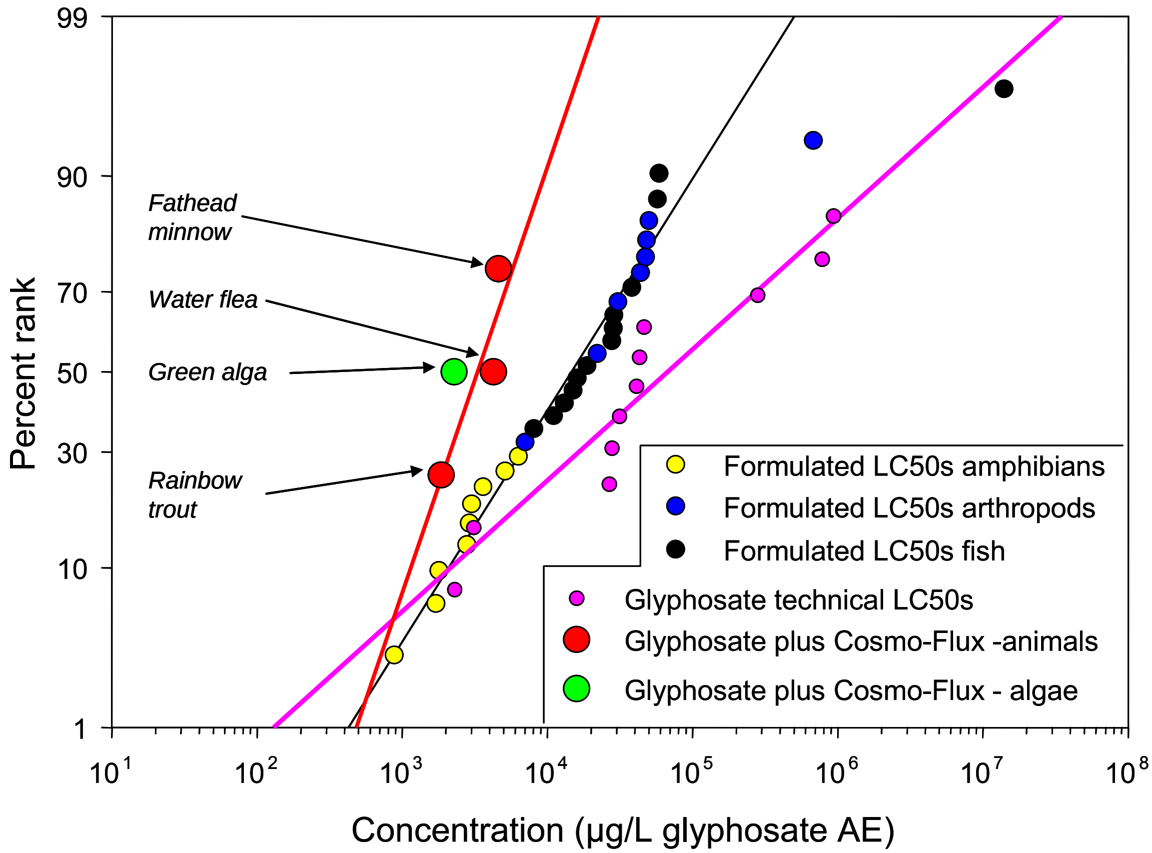


Figure 24 Distribution of toxicity values for glyphosate technical, formulated glyphosate (Roundup®) in all aquatic organisms and in fish and the glyphosate and Cosmo-Flux® 411 mixture as used in Colombia

It is interesting to note that larval amphibians appear to be more susceptible to glyphosate formulation than are other aquatic animals. The reason for this is likely the surfactants in the formulation of Roundup®; as discussed above, other formulations of glyphosate are less toxic to amphibians (Howe et al. 2004).

4.1.3.3 Effects of glyphosate on 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). Leaf absorption 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 dose of glyphosate (Ferreira et al. 1997). This study also indicated that defoliation of *E. coca* 24 hours prior to application resulted in no significant effect of glyphosate (applied up to 6.7 Kg/ha) on regrowth. This 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 is 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, (e.g. Thompson et al. 1997). Studies of glyphosate efficacy on annual weeds have indicated that application during the day (09:00 and 18:00h) 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.

Non-target impacts of glyphosate on seed germination and growth characteristics of the F1 generation of treated wild plant species have been reported. Blackburn and Boutin (2003) noted effects on seven out of 11 species tested with 1%, 10% or 100% of a 0.89 Kg a.i./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 of glyphosate at 1.760 Kg a.i./ha for *Cirsium arvense* control. Nevertheless, applications of glyphosate 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 is 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 (that flower once and die, especially annuals) dependent on seeds for recruitment.

4.2 SURFACTANTS

There are a number of formulations of glyphosate on the market and these may contain a number of surfactants (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; the active ingredient is of very low toxicity to non-target organisms, thus making the surfactant toxicity more important in the risk assessment process. 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. An effect noted with the formulation Gliz 480CS® was caused by non-glyphosate components of the formulation (da Silva et al. 2003). Technical grade glyphosate at concentrations of 52 mM (870 mg/L) did not affect the protozoans *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 eradication of coca and poppy in Colombia contains surfactants as part of the 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. This was the reason why standardized toxicity tests for mammals and environmental non-target organisms were conducted with the spray mixture itself. These are discussed below.

4.2.1 Effects on glyphosate and Cosmo-Flux® on non-target aquatic organisms

A base set of toxicity data is required for all pesticide registrations. For freshwater environments, the set normally makes use of a coldwater fish such as rainbow trout fingerlings (*Onchorynchus mykiss*), a warmwater fish such as fathead minnows (*Pimephales promelas*), an invertebrate such as the water flea (*Daphnia magna*), and an alga such as *Selanastrum capricornutum*. 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 (Table 4), was selected. This mixture contains more Cosmo-Flux® than used for coca and thus represents a worst-case exposure. These data are summarized in Table 19 and Figure 17, above.

4.2.2 Effects of glyphosate and Cosmo-Flux® on mammals

Two series of mammalian toxicity tests on the formulation of glyphosate and Cosmo-Flux® as used for eradication of coca in Colombia were conducted. One set of these studies was conducted in the USA under good laboratory practices (GLP) and using the quality control assurance as appropriate for regulatory decision making. The other studies were conducted in Colombia, also in compliance with GLP and according to US EPA guidelines.

4.2.2.1 Analysis of the formulation

The objective of this study was to assess the concentration(s) of glyphosate (active ingredient) in the formulation (Springborn 2003a). Three 500 mL samples of each mixture were collected from the top/middle/bottom of Air Tractor N8513Q PNC 4003 (Test Article Mixtures 1 and 3), Air Tractor N8514G PNC 4005 (Test Article Mixtures 2 and 4), and Air Tractor N8513V PNC 4004 (Test Article Mixture 5). Test Article Mixtures 1 and 2 were prepared as follows:

Ingredient	Amount Added (gallons)
Herbicide: glyphosate	131.7
Surfactant: Cosmo Flux-411F	3.0
Lake Water	165.3
Mixing Time: Test Article Mixture 1 - 13 minutes; Test Article Mixture 2 - 12 minutes.	

Test Article Mixtures 3, 4 and 5 were prepared as follows:

Ingredient	Amount Added (gallons)
Herbicide: glyphosate	110.0
Surfactant: Cosmo Flux-411F	2.5
Lake Water	137.5
Mixing Time: Test Article Mixture 3 - 12 minutes; Test Article Mixture 4 - 11 minutes; Test Article Mixture 5 - 13 minutes.	

The test article mixtures were prepared on December 5, 2002. The overall concentration of the formulation was 16.53 [in terms of % glyphosate (AE)] before use at SLI and 15.20 [in terms of % glyphosate (AE)] after use at testing laboratory, indicating that the test material was stable during the period of testing. The overall result (16.53% glyphosate AE) was higher than the anticipated 14.80% glyphosate (AE) value but within acceptable error of mixing conditions in the field. Since the results of the analysis were appropriate and would provide conservative results for toxicity, irritation and sensitization because they were slightly higher than expected, the five test article mixes were pooled into a single container for use in the remaining studies.

4.2.2.2 Acute oral toxicity

The single-dose oral toxicity of glyphosate and Cosmo-Flux® was carried out in Sprague Dawley rats (Springborn 2003b). A limit test was carried out in which one group of 10 young adult rats (5 male and 5 female) weighed 325-356 g and 190-208 g respectively and received the test article at a single dose of 5,000 mg/kg body weight (bw). Following dosing, the rats were observed daily and weighed weekly. All animals were humanely killed 14-days post-exposure and subjected to a gross pathology examination. No mortality occurred during the study. Clinical abnormalities observed during the study included transient incidences of soft stools, fecal staining, rough coat, congested breathing, rales (wet, crackly lung noises heard on inspiration which indicate fluid in the air sacs of the lungs), and dark material around the facial area. Body weight gain was noted for all animals during the test period. No significant macroscopic findings were observed at necropsy on study day 14. The oral LD50 for test article in rats was estimated to be greater than 5,000 mg/kg.

Other rat oral acute studies were performed on a mixture of glyphosate (44%), Cosmo-Flux® (1%), and water (55%) (Immunopharmos 2002a) and a mixture of glyphosate (5%), Cosmo-Flux® (1%), and water (95%) (Immunopharmos 2002b).

Both studies were performed according to using EPA guidelines 870-1100. In the first, groups of 5 male and 5 female Wistar rats, approx. 135 g bw, were treated with the test substance by *gavage* at concentrations of 1,250, 2,500 and 5,000 mg/kg bw (Immunopharmos 2002a). The test substance was dissolved in distilled water. The animals were observed for 5 hours during the first day and later on all days during the 14 day post-dosing period. During the study, the animals did not show any adverse effects. The Reed and Muench test was used for the calculation of LD50. The LD50 value of test substance was greater than 5,000 mg/kg bw for males and females.

In the second study (Immunopharmos 2002b), groups of 10 Wistar rats (5 male and 5 female), ranging from 116 to 138 g bw, were treated with the test substance by *gavage* at concentrations of 1,250, 2,500 and 5,000 mg/kg bw. The test substance was dissolved in distilled water. The animals were observed as above. During the study, the animals showed no adverse effects. The Reed and Muench test was used for the calculation of LD50. The LD50 value of test substance was greater than 5,000 mg/kg bw for males and females.

4.2.2.3 Acute Inhalation toxicity

A limit test was performed in 10 young adult Sprague Dawley rats (5 male and 5 female) weighing 248-275 g and 201-212 g respectively received a 4-hour nose-only inhalation exposure at an aerosol concentration of 2.60 mg/L (Springborn 2003c). The mass median aerodynamic diameter and geometric standard deviation of the sampled particles were 2.9 µm ± 2.17. The percentage of particles ≤ 4.0 µm was determined to be 66%. After exposure, the rats were observed daily and weighed weekly. All animals were humanely killed at 14-days post-exposure and subjected to a gross pathology examination on day 14. There was no mortality during the study. The clinical abnormalities observed during the study included breathing abnormalities, no/decreased defecation, urine staining, rough hair coat, dark material around the facial area and decreased food consumption. Body weight loss was noted in 2 males and 1 female during days 0 to 7. Body weight gain was noted for all other animals during the test period. At study termination, the animals had exceeded/maintained their initial body weight. No macroscopic findings were observed at necropsy (day 14). The inhalation LC50 of test material was estimated to be greater than 2.60 mg/L but exposures greater than or equal to this value may be harmful.

Other rat acute inhalation toxicity studies were performed on a mixture of glyphosate (44%), Cosmo-Flux® (1%), and water (55%) (Immunopharmos 2002a) and a mixture of glyphosate (5%), Cosmo-Flux® (1%), and water (95%) (Immunopharmos 2002b).

Both studies were performed under EPA guideline 870-1300. In the first, ten Wistar rats (5 male and 5 female) were used for each concentration (Immunopharmos 2002c). The test substance was dissolved in sterile water to achieve concentrations of 5, 10, and 20 mg/L air/hour during 4 hours of exposure. After the exposure period, the animals were kept for a 14-day observation period. The mass median aerodynamic diameter and geometric standard deviation of the sampled particles were not indicated. There were no deaths during exposure period and no signs of systemic toxicity were observed at the three concentrations tested. All animals were humanely killed at 14 days post-exposure and subjected to a gross pathology and histopathology examinations and no abnormalities were observed. The LC50 value of the test substance was higher than 20 mg/L of air. Therefore, the test substance is not considered as harmful at concentrations less than 20 mg/L.

In the second study (Immunopharmos 2002d), ten Wistar rats (5 male and 5 female) were used for each concentration. The test substance was dissolved in sterile water to achieve concentrations of 5, 10, and 20 mg/L air/hour during 4 hours of exposure. After the exposure period, the animals were kept for a 14-day observation period. The mass median aerodynamic diameter and geometric standard deviation of the sampled particles were not indicated. There were no deaths during the exposure period and no signs of systemic toxicity at the three concentrations tested. All animals were humanely killed 14 days post-exposure and subjected to a gross pathology and histopathology examinations. At necropsy the surviving animals showed petechial lung (3/10) while the remaining organs were normal. The LC50 value of the test substance was higher than 20 mg/L of air.

4.2.2.4 Acute dermal toxicity

A limit test was performed in 10 Sprague Dawley rats (5 male and 5 female) receiving a single dermal administration of the test article at a dose of 5,000 mg/kg bw (Springborn 2003d). Following dosing, the rats were observed daily and weighed weekly. All animals were humanely killed after 14-days exposure and subjected to a gross pathology examination. No mortality occurred during the study. Clinical abnormalities observed during the study included transient incidences of dark material around the facial area and decreased defecation. Dermal irritation was noted at the site of test article application. Body weight loss was noted in 1 male and 2 females during the study (day 7 to 14). Body weight gain was noted for all other animals during the test period. At necropsy (day 14), no significant macroscopic findings were observed. The acute dermal LD50 of test article was estimated to be greater than 5,000 mg/kg in the rat.

4.2.2.5 Skin irritation

A potential irritation of the test material was evaluated on the skin of New Zealand White rabbits (Springborn 2003e). Each of 3 rabbits (13 weeks of age and weighed 2.5-2.8 kg prior to dosing) received a 0.5 ml dose of the test article as a single dermal application. The dose was held in contact with the skin under a semi-occlusive binder for an exposure period of 4 hours. Following the exposure period, the binder was removed and the remaining test article was wiped from the skin using gauze moistened with deionized water followed by dry gauze. Test sites were subsequently examined and scored for dermal irritation for up to 72 hours following patch application. Exposure to the test article produced very slight erythema on 3/3 test sites at the 1-hour scoring interval. The dermal irritation resolved completely on all test sites by 24-hour. The test article was considered to be a slight irritant to the skin of the rabbit. The calculated Primary Irritation Index for the test article was 0.25.

Other skin irritation studies were performed on a mixture of glyphosate (44%), Cosmo-Flux® (1%), and water (55%) (Immunopharmos 2002g) and a mixture of glyphosate (5%), Cosmo-Flux® (1%), and water (95%) (Immunopharmos 2002h). Both studies were performed using EPA guidance 870-2500.

In the first, 0.5 ml of test substance was applied to the clipped and abraded skin of 3 male and 3 female New Zealand White rabbits (2.3-2.4 kg bw) (Immunopharmos 2002g). The application site of the test substance was covered with three occlusive dressings for 15 minutes, 1 hour, and 4 hours, after which the site was washed. Skin reactions were measured for erythema and edema using a modified Draize test. The readings were made at 24, 48, and 72 hours after treatment. Body weight was not measured. There were no signs of irritation at the application site or systemic toxicity. In the second study, 0.5 ml of test substance was applied to the clipped and abraded skin of 3 male and 3 female New Zealand White rabbits (2.3-2.4 kg bw) (Immunopharmos 2002h). The application site of the test substance was covered with three occlusive dressings for 15 minutes, 1 hour, and 4 hours, after which the site was washed. Skin reactions were measured for erythema and edema using a modified Draize test. The readings were made at 24, 48, and 72 hours after treatment. Bodyweight was not measured. There were no signs of irritation and/or edema on the shaved skin.

4.2.2.6 Eye irritation

The eye irritation for the test article was evaluated in rabbits (Springborn 2003f). Each of 3 New Zealand White rabbits received a 0.1 mL dose of the test article in the conjunctival sac of the right eye. The left eye of each untreated animal served as a negative control. Test and control eyes were examined for signs of irritation for up to 7 days after dosing. Exposure to the test article produced iritis (3/3 test eyes) at the 1-hour scoring interval which resolved completely in all eyes by 24-hour. Conjunctivitis (redness, swelling and discharge) was noted in 3/3 test eyes at the 1-hour. The conjunctival irritation resolved completely in all treated eyes by day 7. An additional ocular finding of slight dulling of normal luster of the cornea was noted in 1/3 test eyes. Based on these results, the test material is considered to be a moderate irritant to the eye.

Other eye irritation studies were performed on a mixture of glyphosate (44%), Cosmo-Flux® (1%), and water (55%) (Immunopharmos 2002e) and a mixture of glyphosate (5%), Cosmo-Flux® (1%), and water (95%) (Immunopharmos 2002f). Both studies were performed using EPA guidance 870-2400.

In the first, 18 New Zealand White rabbits were used (Immunopharmos 2002e). The test substance (0.1 ml) was placed into the conjunctival sacs of rabbits. The left eye of each untreated animal served as negative control. The eyes of 3 rabbits of each sex were rinsed for 30 second after the test substance application. A further 6 rabbits were left with unrinsed eyes. The eyes were examined for irritation at 1, 24, 48, 72, 96 hours, and 7 days after instillation. The animals showed the following signs: opacity (5/12, from grade 1 to 3); corneal damage (4/12 neovascularization on cornea); iritis (5/12 grade 1, disappearing 4 days latter); conjunctivitis (12/12 from grade 1 to 3); chemosis (10/12 from grade 1 to 3); discharge (4/12 animals presented discharge the first days of the study).

The eyes of the 6 animals rinsed 30 seconds after application of the test substance presented as follows: opacity (6/6 did not present corneal opacity); corneal damage (6/6, with no damage); iritis (6/6 with no iritis); conjunctivitis (6/6 animals presented from grade 1 to 3, which was diminishing which disappeared at the end of the study, 7 days); chemosis (3/6 animals presented grade 1 which disappeared in 24 hours); discharge (6/6 animals presented discharge the first two days of the study). In conclusion, the test substance caused slight to moderate irritation in the eyes from animal that were treated and then not rinsed. This irritation was observable between days 1 and 7. In contrast, the test substance did not produce irritation in animals, the eyes of which were treated and then rinsed for 30 seconds after the application of test substance.

In the second study, 18 New Zealand White rabbits were used (Immunopharmos 2002). Again, 0.1 ml of the test substance was placed into the conjunctival sacs of rabbits. The left eye of each untreated animal served as negative control. The eyes of 3 rabbits of each sex were rinsed for 30 seconds after the test substance application. A further 6 rabbits were left with unrinsed eyes. The eyes were examined for irritation at 1, 24, 48, 72, 96 hours after instillation. The test substance did not cause irritation in the eyes from animals treated and not rinsed (observed between days 1 and 4). The test substance did not produce irritation in the eyes of animals treated and rinsed 30 seconds after the application of test substance and then observed for 4 days.

4.2.2.7 Skin sensitization

The dermal sensitization potential of test substance was evaluated in guinea pigs (Springborn 2003g). Twenty Hartley albino guinea pigs (10 male and 10 female) were topically treated with 100% test substance, once per week, during three weeks. Following a 2-week rest period, a challenge was performed [20 animals treated and 10 animals untreated (challenge control)] were topically treated with 100% test substance. A positive control group was given hexylcinnamaldehyde (HCA). Based on the results of this study, test substance was not considered to be a sensitizer.

Other skin sensitization studies were performed on a mixture of glyphosate (44%), Cosmo-Flux® (1%), and water (55%) (Immunopharmos 2002j) and a mixture of glyphosate (5%), Cosmo-Flux® (1%), and water (95%) (Immunopharmos 2002i). Both studies were performed according to EPA guideline 870-2600. In the first, 30 Hartley guinea-pigs (300-350 g bw), were divided into 6 groups; 2 groups of males with 5 animals and 2 groups of females with 5 animals for the study, and 2 groups of 5 animals of both sexes that serves as control. The test substance (0.5 ml) was applied to the skin of albino guinea-pigs three times with an interval between each exposure of 1 week (0, 7, and 14 days) and for a duration of 6 hours in each application. The animals were inspected at 24, 48, and 72 hours after applications. The control group (5 male and 5 female) received sterile distilled water. A positive sensitization study was conducted every 6 month using a sensitizing agent (data not given). The test material caused no dermal adverse reactions even after several applications (Buehler test). It was noted that the test material was not a sensitizer for the skin

In the second study (Immunopharmos 2002i), 30 Hartley guinea-pigs (300-350 g of weight), were divided in 6 groups; 2 groups of males with 5 animals and 2 groups of females with 5 animals for the study, and 2 groups of 5 animals of both sexes that served as a control. The test substance (0.5 ml) was applied to the skin of albino guinea-pigs, three times with an interval for each exposure of 1 week (0, 7, and 14 days) and 6 hours for each application (Buehler test). A total of 0.5 ml was applied over the exposed skin. The animals were inspected at 24, 48, and 72 hours after application. The control group (5 male and 5 female) received sterile distilled water. The positive sensitization study was conducted in the laboratory every 6 months using a sensitizing agent (data not given). The test material caused no adverse dermal reactions even after several applications (Buehler test). It was concluded that the test material was not a sensitizer for the skin.

4.2.2.8 *General conclusions on the mammalian acute toxicity of glyphosate and Cosmo-Flux®*

Based on the results of these studies undertaken with the mixture glyphosate and Cosmo-Flux®, the following conclusions can be drawn:

- The acute oral and dermal LD50 value was estimated to be greater than 5,000 mg/kg bw in the rat. Therefore, this formulation is considered as practically non-toxic by the oral route.
- The acute inhalation LC50 value was estimated to be greater than 2.60 mg/L in the rat. In one study the rats showed breathing abnormalities after exposures at 2.6 mg/L for 4 hours. This value for the test substance is considered as potentially harmful for durations of exposure of the order of 4 hours. In two other studies, the mixture was shown to not be harmful at exposures up to 20 mg/L for 4 hours. Exposures via the inhalation route in these animal 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 hazard to the 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 the glyphosate did not change its toxicological properties to mammals.

4.3 EFFECTS IN THE FIELD

4.3.1 Duration of effects in the field

In tropical forest situations, similar to some of the locations of the 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 been a historical feature of the development of agriculture from across the globe, (e.g. Boahene 1998, Matlack 1997). In Central America, agricultural intensification and forest clearance in Mayan and other cultures has been determined from the pollen record, (e.g. 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 note:

> "*the consensus of these analyses is 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. Nevertheless, the recovery of biophysical properties and vegetation is heavily dependent on the interactions between site-specific factors and*

land use, which makes 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). While 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 understorey vegetation in managed northern forests, so-called conifer-release treatments, (e.g. Lautenschlager and Sullivan 2002). Recovery of the deciduous herb and shrub layers occurs over a period of 2-3 years in general and the tree layer over 10 years (See Section 4.3.2.3). Often, total structural diversity is unaffected by glyphosate treatments after one year.

4.3.1.1 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.

Whilst vegetation recovery may be rapid, in eastern North America, research has led to the surprising conclusion that 19^{th} 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.

4.3.1.2 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 suggest that 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, though 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 years (Williams et al. 2001).

Whilst 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 indeed with eradication areas.

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 years and 7-11 years post application (Eschenburg et al. 2003, Eschholz et al. 1996). At 1 and 2 years post-treatment, tracks of foraging moose were 57 and 75% less abundant on treated than untreated clear-cuts ($P = 0.013$). However, at 7-11 years post-treatment, 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 years post-treatment 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 greater counts of tracks of foraging moose on older treated clear-cuts was due to 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 years. Studies of small mammal responses to glyphosate vegetation control in similar environments (Sullivan et al. 1998) have indicated that vegetation recovery 2-3 years 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 (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.

4.3.1.3 Interactions with surfactants

Surfactants significantly improve coca control with glyphosate (Collins and Helling 2002) and control of *Salvinia molesta*, an aquatic fern (Fairchild et al. 2002). Nevertheless, the behavior of surfactants is complex (Liu 2004). Spray droplet size affects retention on the target plant, but also the absorption into the plant. Smaller droplets are better retained on the plant, but absorption through the leaf is better from larger "coarse" droplets (Feng et al. 2003). A study of volume rate effects of glyphosate on grasses has shown that reduced application volumes give better control, partly affected by the concentration of surfactants in formulated products (Ramsdale et al. 2003).

Studies of biodegradable non-phytotoxic rapeseed oil derivatives (triglyceride ethoxylates; Agnique RSO(R) series containing an average of 5, 10, 30 and 60 units of ethylene oxide) indicate that these adjuvants gave similar or better control of *Phaseolus* *vulgaris* L. compared with 0.36 Kg AE/L SL Roundup Ultra®. In these studies Agnique RSO 60 generally was most effective (Haefs et al. 2002). Tests with a range of surfactants and different herbicides on several plant species indicate that the optimum surfactant structure is both herbicide and plant species dependent (Johnson et al. 2002).

Studies of synergism between amino acid biosynthesis-inhibiting herbicides indicate that, in most cases associated with glyphosate, the lack of effects with technical herbicide confirm that surfactants are important components of formulated products (Kudsk and Mathiassen 2004).

4.3.2 Recovery from effects

4.3.2.1 Principles

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 a period of 2 to 3 weeks, though species with extensive storage organs, e.g. long rhizomes, large size, or particularly impenetrable leaf surfaces, may survive. A low 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, pre-harvest weed control in annual crops and for the eradication 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 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.

4.3.2.2 Tropical situations

In tropical forests, similar to some of the locations of the coca eradication programs, there is 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 y) 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 y) 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 et al. 2004, Martins and Rodrigues 2002). 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 gap dynamics; this deserves further scientific study.

In the high Andes alpine paramo habitats, patterns of succession were described (Sarmiento et al. 2003). Following cultivation, usually for potato, patterns of secondary succession were such that after 12 years, the 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 low, 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 program areas.

4.3.2.3 Temperate situations

Glyphosate has been widely used for controlling deciduous understorey vegetation in managed northern forests, so-called conifer-release treatments, (e.g. Lautenschlager and Sullivan 2002). Effects on the successional patterns of vegetation in such temperate and boreal situations are that woody and herbaceous species are most reduced by glyphosate, (e.g. Bell et al. 1997). In a study in British Colombia, 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 ($p>0.10$) different 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 the species richness, diversity, turnover, or structural diversity of the plant community. The authors note that the 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 years 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, Sullivan et al. 1998). Nevertheless, some plant groups may take longer to recover from glyphosate application. For example, cryptogams (ferns) may take longer than 5 years to recover in boreal forest situations (Newmaster and Bell 2002), probably reflecting longer generation times and poor dispersal. 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. Reviewing the effects of glyphosate use in forestry, (Sullivan and Sullivan 2003) noted that:

> "…*the magnitude of observed changes in mean species richness and diversity of vascular plants, birds, and small mammals, from the effects of herbicide treatment, were within the mean values of natural fluctuations of these variables. The biological significance of this impact is limited to shifts in species composition based on changes in floral composition and structure of habitats. Management for a mosaic of habitats within forest and agricultural landscapes, which provide a range of conditions for plant and animal species, should help ameliorate the short-term changes in species composition accompanying vegetation management with glyphosate*".

Single applications of glyphosate control much of the vegetation that receives spray, but recovery is generally rapid and within the range of natural disturbances.

4.3.2.4 Conclusions

The experience of glyphosate use in northern temperate forests is such that vegetation and fauna recover over a period of 2 to 3 years, 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 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). Whilst 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. Re-application frequencies are generally greater than 6 months for coca and greater than 3 months for poppy and, bearing in mind the molecule is biologically unavailable in the soil and soil-bound residues have a half life of between 14 and 32 days, the environmental impacts are no greater than single applications.

5 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 US EPA (1993b). The estimated exposures used were those calculated for the use of glyphosate for eradication spraying in Colombia. This was done for human and environmental risks and is outlined above.

5.1 HUMAN HEALTH

From an assessment of the results of toxicity testing of the formulation of glyphosate and Cosmo-Flux® as used in Colombia (Section 4.2.2), it was concluded that the addition of Cosmo-Flux® to the spray mixture did not affect the 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 7-9. The greatest values were taken as reasonable worst-case for a hazard assessment. These results are shown in Table 20 and illustrated graphically in Figure 18. In comparing the exposure and effect concentrations a margin of exposure approach was used. Thus a number greater than 1 (Table 20) means that the exposure was less than the exposure or dose that caused the response in the toxicology study.

From the data in Table 20, it is clear that potential exposures to glyphosate and Cosmo-Flux® as used for the eradication of coca and poppy in Colombia 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 US EPA. The toxicity values used in both of these approaches were derived from chronic exposures where the animals were dosed over extended time periods. They are thus 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, but as discussed above, droplet size is large and inhalation will be less than in the laboratory animal studies as well as the droplet size used in agricultural uses, from which the potential inhalation exposure was derived.

Table 20. 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.

Source of exposure	**Exposure value in mg/kg**		**Margin of exposure compared to the most sensitive NOEL(175 mg/kg)**	
	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
Source of exposure	Exposure value in mg/kg		Margin of exposure for the US EPA RfD (2 mg/kg/day)	
	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

5.2 ENVIRONMENT

Assessment of the environmental risks of glyphosate and Cosmo-Flux® to aquatic organisms was based on data from the literature and from studies conducted on the mixture of formulated glyphosate and Cosmo-Flux® as used in Colombia. As discussed in Section 4.1.2, the toxicity of the mixture of glyphosate and Cosmo-Flux® was greater than that reported for formulated glyphosate itself. When the toxicity values for the mixture as used in Colombia are compared to the range of estimated exposures that would result from a direct overspray of surface waters (Table 10), it is clear that aquatic animals and algae in some shallow water bodies may be at risk (Figure 19).

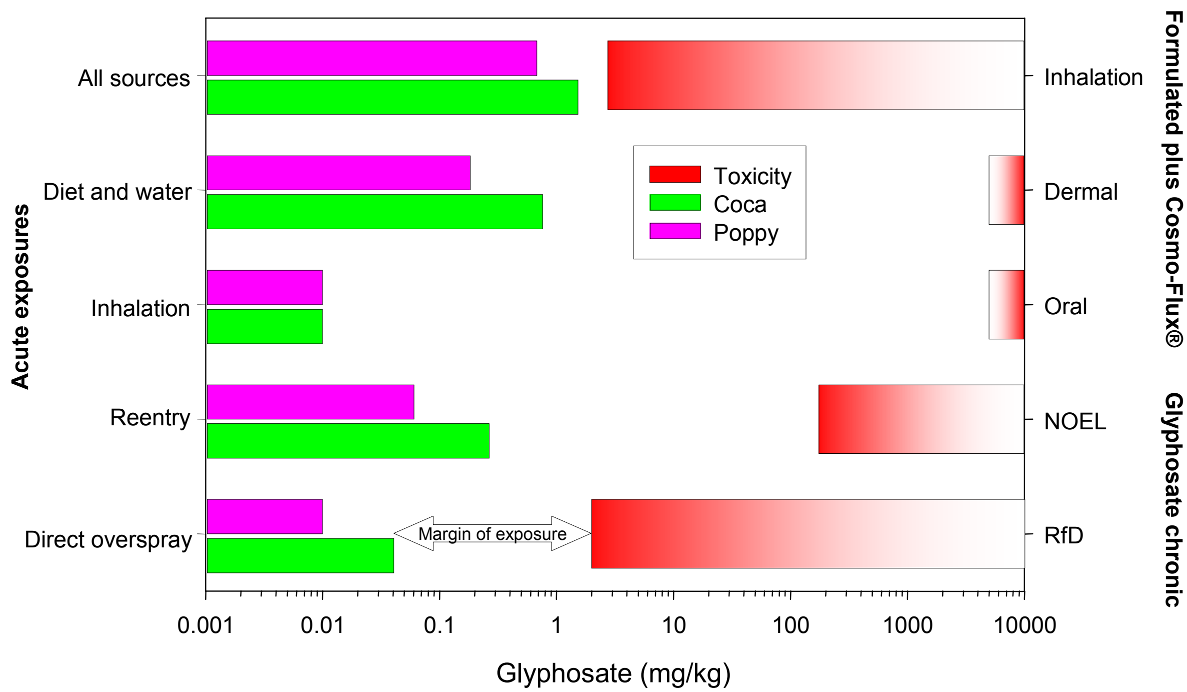


Figure 25 Illustration of acute toxicity values in laboratory mammals for glyphosate plus Cosmo-Flux®, the NOEL from the most sensitive chronic study in laboratory animals, and the RfD (glyphosate) and the estimated worst-case acute exposures that may be experiences under conditions of use in Colombia.

While the overlap of the range of estimated exposure concentrations with the toxicity values for the green alga and rainbow trout suggests 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 in close enough proximity to the coca field that it is accidentally over-sprayed, that it is less than 30 cm deep, and that it is not flowing. Because flow of the water would likely result in rapid hydraulic dilution to concentrations to below the threshold of biological activity, organisms in flowing water would not be at risk. It was not possible to determine the actual frequency of these risks as data on proximity of surface water to coca fields is not available at this time. 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 yet been conducted on the mixture of glyphosate plus Cosmo-Flux® as used in Colombia.

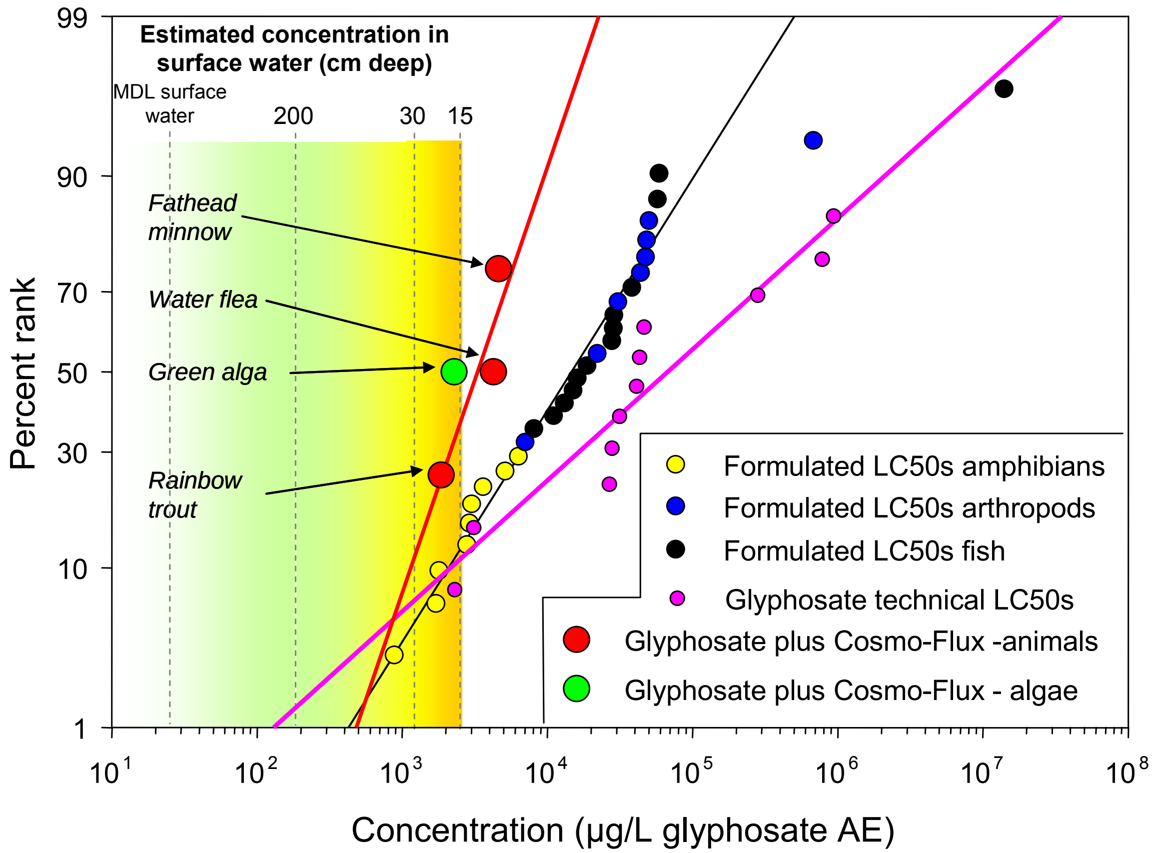


Figure 26 Distribution of toxicity values for glyphosate technical, formulated glyphosate (Roundup®) in all aquatic organisms and in fish and the toxicity values in four aquatic species for glyphosate and Cosmo-Flux® 411 mixture as used in Colombia. The yellow rectangle shows the range of predicted worst-case exposures resulting from direct overspray of surface waters ranging from 15 to >200 cm in depth. Lines are the regressions through the log-probability transformed data.

Based on the toxicity data for honeybees (Section 4.1.2.1), the mixture of glyphosate and Cosmo-Flux® 411F is not acutely toxic via contact exposure to honey bees. It did not cause mortality or stress effects in bees in the normal 48 hour period after treatment at concentrations equal to or less than 56.8 mg AE/bee. These results show that the formulated product is not directly hazardous to bees and, by extrapolation, to other beneficial insects.

Although no acute or chronic data are available on wild animals, extrapolation of the mammalian data discussed above (Sections 4.1.2 and 4.2.2) and from reports in the literature support the conclusion that glyphosate and Cosmo-Flux®, as used in the eradication program in Colombia, 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 and harvesting the crop 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 over-sprays that affect non-target vegetation, however, as discussed above (Section 2.1.3.5), these areas are small in relation to the sprayed fields < 0.48%), represent a very small proportion of the total habitat available << 0.001%, and will undergo rapid recolonization and succession to habitats suitable for wildlife.

6 CONCLUSIONS

Because of differences in the approaches to human and ecological risk assessment, the conclusions of this report are discussed separately in the following sections. In these discussions, the 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 as discussed in the Problem Formulation (Section 2.2.1).

6.1 HUMAN HEALTH RELEVANCE

Based on all of the evidence and information presented above, the Panel concluded that the risks to humans and human health from the use of glyphosate and Cosmo-Flux® in the eradication of coca and poppy in Colombia were minimal (Figure 20). The 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 to other risks associated with clearing of land, the uncontrolled and unmonitored use of other pesticides 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.

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	**55.5**
Eradication spray	**<0.1**	**0**	**1**	**<0.1**	**<0.1**
Processing and refining	5	3	5	75	**27.8**

Figure 27 Potential human health impacts of the cycle of coca or poppy production and the spray eradication program.

6.2 ECOLOGICAL RELEVANCE

Based on the evidence and data discussed above and the results of a number of specific studies conducted specifically for this assessment, the Panel concluded that the risks to the environment from the use of glyphosate and Cosmo-Flux® in the eradication of coca and poppy in Colombia were small in most circumstances (Figure 21). 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 over-sprayed. 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.

Coca or poppy field developed in a natural area

IMPACTS	INTENSITY SCORE	RECOVERY TIME (Y)	IMPACT SCORE	% IMPACT
Clear cutting and burning	5	60	300	**97.6**
Planting the coca or poppy	1	4	4	**1.3**
Fertilizer inputs	1	0.5	0.5	**0.2**
Pesticide inputs	2	0.5	1	**0.3**
Eradication spray	**1**	**0.5**	**0.5**	**0.2**
Processing and refining	2	1	2	**0.7**

Figure 28 Potential environmental impacts of the cycle of coca or poppy production and the spray eradication program.

6.3 STRENGTHS AND UNCERTAINTIES IN THE ASSESSMENT

This assessment has both strengths and uncertainties. These are discussed in the following sections. These strengths and uncertainties lie in the exposure and effects characterizations and, because these 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 of this report.

6.3.1 Exposures

6.3.1.1 Environmental exposures

Applications of glyphosate are well characterized. State of the art equipment is used. The locations of application and the areas sprayed are well documented and measured with resolutions only equaled in some applications in forestry in other jurisdictions. The mixing and application rates are well characterized and the probability of application of amounts of glyphosate and Cosmo-Flux® greater than those specified are judged to be small. The resultant 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 in the eradication program in Colombia, will not persist, accumulate, or biomagnify in the environment. 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 environments where it is applied. Residues of glyphosate were not frequently 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 non-eradication 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 to these surface waters. The sampling of the surface waters only took place for a period of 24 weeks and only 5 locations were sampled in this way. Although two of these were scheduled to be sprayed, only one location was treated during the sampling period. For logistical reasons, it was also not possible to sample close to the application sites. If sampling had been conducted at more sites closer to the sprayed fields and over a longer time period, residues may have been detected more frequently.

6.3.1.2 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 over-sprayed. Exposures were judged to be small and, in all cases, considerably below thresholds of concern.

Application rates of glyphosate used for coca eradication 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 considerably below the US EPA reference dose.

6.3.2 Effects

6.3.2.1 Environmental effects

The environmental toxicology database for glyphosate is relatively large and its effects in non-target organisms are well known or can be extrapolated. Glyphosate itself is of low toxicity to non-target organisms, however, there are a number of formulations of glyphosate that exist in the marketplace and these may contain many different surfactants and/or adjuvants. It is also known that it is the surfactants that determine the toxicity of the formulation as many are more toxic than technical glyphosate itself. Because of this, the Panel had several toxicity tests conducted with the formulated product of glyphosate plus Cosmo-Flux® as used in the eradication program in Colombia. This reduced uncertainty with respect to toxicity to beneficial insects such as the honeybee and to aquatic organisms. Recent studies have reported that amphibians, such as frogs, are amongst the more sensitive aquatic organisms with respect 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.

6.3.2.2 Effects in humans

The database of effect data for glyphosate is large and its risks to humans and the environment have extensively reviewed and assessed in a number of national and international jurisdictions as well as in the open scientific literature. In all cases, glyphosate has been judged to be of low risk. However, some of the studies on which these assessments are based were conducted prior to 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 re-registration, older studies will be replaced with newer tests conducted according to current guidelines. Given the large and expanding use of glyphosate in agriculture, the 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. The Panel also conducted a preliminary epidemiological study to assess possible linkages between the use of glyphosate and adverse human-health outcomes and 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 of this study 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.

6.3.3 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, the Panel recognizes 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 eradication program in Colombia. Exacerbating these hazards is the lack of information about the conditions of use of these substances. 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. This represents a significant and serious potential risk to humans and the environment.

6.4 RECOMMENDATIONS

The Panel has identified a number of uncertainties in its review of the data and from these makes the following recommendations. These recommendations are grouped into two classes, recommendations to retain current practices that were judged to be essential or useful (Table 21) and recommendations related to new activities or data collection that will address key uncertainties identified in our study (Table 22).

Table 21. 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 modification of current procedures 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 lower than any currently-available alternatives. However, if new candidate products become available, their use should only be considered after an appropriate risk assessment has been conducted.	4

Table 22. 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 non-flowing 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 non-target 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
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	2

Table 22. 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)
	its use in eradication spraying in Colombia.	
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 lower environmental toxicity.	Better characterization of exposures under conditions of use in Colombia.	1

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8 GLOSSARY

Absorption: The movement of a substance across an exposed surface (e.g., skin, respiratory / digestive mucous) and into the circulation to be distributed throughout the body. This will vary depending on a compound's inherent ability to cross a particular barrier.

AE - Acid Equivalent: The concentration of a substance (glyphosate) expressed in terms of the amount of glyphosate acid, rather than the salt.

A.I. - Active Ingredient: The component of a mixture / formulation which is ultimately responsible for the physiological effects.

Acute toxicity: The potential of a compound to cause injury or illness when given in a single dose or in multiple doses over a short period of time (e.g. 24 h). These effects are based on mechanisms of chemical action where perceptible physiological alterations can be appreciated shortly after administration (e.g. death).

ADI - Acceptable daily intake: This is an estimate of the maximum amount of a compound (often in food) which can be ingested daily over a lifetime without any appreciable detrimental health effects. This parameter has been developed primarily by the WHO and FAO.

Adjuvant: Ingredient added to a particular formulation in order to enhance the availability and efficacy of the active ingredient. These often act by increasing the spreading or uptake of the active ingredient(s).

Adsorption: The process by which a compound is held or bound to a surface by chemical or physical attraction.

Anthropogenic: Chemicals artificially developed by man.

Aromatic: Organic compound in which constituent atoms form a ring (s). These ring structures may grant a compound its characteristic properties such as solubility in lipids.

Bioaccumulation: The accumulation of a particular compound in certain body tissues. This occurs when rate of uptake exceeds that of metabolism and/or excretion. Over time this results in a higher concentration of the substance in the organism than in its environment. Important factors governing the extent of this process include the lipid solubility of the compound as well as how readily it is metabolized.

Bioactivation: The process by which a chemical becomes more reactive due to alterations in its structure and hence chemical properties. This can occur in the environment or within a biological system.

Bioconcentration Factor (BCF): Measure of the tendency of a substance in water to accumulate within the tissues of fish or other organisms. The concentration in the organism can be roughly calculated by multiplying the concentration in the water by the bioconcentration factor. The value determined is useful in helping to determine the possible human consumption level.

CAS No.: Chemical Abstract System registry number. Pertains to a database providing chemical substance information.

Carcinogenic: Any chemical that can cause the formation of cancerous lesions. Often this is achieved through the formation of genetic mutations within a cell(s) resulting in the loss in ability to regulate proliferation.

Chlorosis: A disease in plants, causing the flowers to turn green or the leaves to lose their normal green color.

Chronic toxicity: The nature of adverse effects over a prolonged period of chemical exposure. Such effect measures can include the development of cancer or decrease in growth.

Dermatitis: Inflammation of the skin.

Dose-response: The change in the intensity of physiological effect with dosage. The relationship of response to dose will vary depending on the mechanism through which the compound is acting.

EC50: Median effective concentration. The concentration of a substance in a medium (such as water) which produces an defined effect in 50% of test organisms.

Ecosystem: A collection of populations (microorganisms, plants, and animals) that occur in the same place at the same time and that can therefore potentially interact with each other as well as their physical and chemical environment and thus form a functional entity.

Emulsification: The mixture of two immiscible (non-mixable) liquids by the dispersion of one into the other in the form of tiny droplets.

Environmental fate: The movement, accumulation, and disappearance of chemicals in the environment after their release.

EPA: Environmental Protection Agency (and in the U.S. EPA).

Epidemiological study: The study of the distribution and determinants of health-related states and events within populations. The prevalence of a particular disease as well as various risk factors for its development are studied.

Exposure: Amount of a chemical which comes into contact with a body surface (skin, respiratory tract, digestive tract) from which it can be absorbed into the body. Exposure does not include any chemical that is nearby but not in contact or which is intercepted by clothing or protective equipment.

Exposure route: The means by which a compound comes into contact an absorptive interface such as dermal or inhalation.

Formulant: A substance normally added to a pesticide to increase its ease of use, penetration into the target organism, or to facilitate its application.

Genotoxic: Describes any substance capable of damaging DNA resulting in mutations or the development of cancer.

Half-life: The time for the concentration of a particular chemical or drug to decrease by half of its initial concentration. This will vary depending on its rate of degradation, metabolism, and/or elimination.

Hazard quotient: The ratio of exposure concentration to a reference (threshold) value. If this value is above acceptable concentration, an adverse effect is possible.

Inert ingredients: All components of a mixture not classified as the primary active ingredient. See, Formulant.

Intraperitoneal: Within the peritoneal cavity, the area that contains the abdominal organs.

Intravenous: The injection or entry of a substance directly into a vein and hence into general circulation.

K_{OW} (Log): The octanol-water partition coefficient (K_{OW}) is a ratio of the concentration of the chemical in n-octanol and water at equilibrium. Chemicals with a K_{OW} greater than 1 preferentially partition into octanol. May be expressed as a $\log_{10}$. The value obtained from this determination gives an indication of the potential for the substance to bioconcentrate into organisms.

LC50 - Lethal Concentration 50: The concentration that is lethal to 50% of test organisms. This value is usually used when referring to the toxicity of a substance to organisms exposed via a matrix such as water.

LD50 – Lethal Dose 50: The dose that is lethal to 50% of test animals. This value is used when referring to the toxicity of a substance to organisms that exposed to a specific amount of substance such as via the oral or the injection route.

Leaching: The movement of a substance through the soil.

LOAEL - Lowest Observed Adverse Effect Level: The lowest dose of a toxin at which an adverse effect can be noted in a particular test species. This value will vary depending on the species being utilized.

Matrix: The medium through which an organism may be exposed to a substance. Water for aquatic organisms, soil for soil organisms, air, etc.

Mechanism of action: The process by which a substance produces its characteristic effects. It is often used interchangeably with “toxic mode of action” however it is usually a more specific term. This is a description of the physiological processes that are altered and the consequences of such changes.

Metabolite: A product of natural metabolic processes.

MRL-Maximum Residue Limit: The maximum amount of a substance permissible on food products as well as animal feeds. This value is recommended by the Codex Alimentarius Commission. This takes into account various safety factors as does the ADI.

MTD-Maximum Tolerated Dose): The dose at which significant toxic effects occur without resulting in death.

Mutagen: Any substance or agent that is capable of creating changes in DNA that are subsequently passed on to future cells. These changes may sometimes lead to the development of cancer or changes in organism characteristics.

NOAEL-No Observable Adverse Effects Level: The highest dose that results in no adverse effects being noted in test organisms.

Oxidation: An alteration of chemical structure by the removal of an electron. This is accomplished by any compound that is capable of achieving this (oxidant).

Percutaneous: Pertaining to any agent than can traverse or is administered through the skin.

Persistence: The resistance of a substance to metabolism or environmental degradation. A chemical deemed as persistent will have a long half-life and will remain in the environment for an extended period of time.

PPB-Parts Per Billion: A measure of concentration where the proportion is such that one part of solute exists per one billion parts of solvent or matrix.

PPM-Parts Per Million: A measure of concentration where the proportion is such that one part of solute exists per one million parts of solvent or matrix.

RfD-Reference Dose: A numerical estimate of a daily oral exposure to humans of a substance. This dose level considered unlikely to cause harmful effects during a lifetime. This value takes into account sensitive subgroups whom can be exposed to this agent.

Safety factor: The difference between the NOAEL and the dose allowed in routine exposure. This value is calculated by using the NOAEL for the most sensitive species and dividing it by various uncertainty factors depending on the readily available scientific data. For example if a value is being extrapolated to man from animals, the NOAEL will be divided by a factor of 10. Such numerical factors will vary depending on the size of the uncertainty (i.e. more related species extrapolation will utilize a smaller safety factor).

Sensitizer: A chemical that is capable of causing the development of an allergic response upon subsequent exposure.

Solubility: The relative ability of a certain substance to be dissolved in a particular solvent. For example, compounds that are very readily dissolved in water may be only minimally dissolved in a more lipid-like solvent such as organic solvents (e.g. octanol).

Sub-chronic: Refers to a period of repeated exposure which is usually about 10% of an organism's expected life-span.

Synergism: The process by which two or more substances interact via a biological mechanism to produce a greater than additive response.

Teratogenesis: The development of a deformed offspring after exposure of the fetus to a certain chemical insult. The various developmental stages at which this exposure occurs will result in different abnormalities.

Toxicity test: The determination of the toxic potential of a particular substance on a group of selected organisms under defined conditions.

Toxicodynamics: The mechanism through which a toxic compound exerts its physiological effect. This includes the relationship between the structure of a compound and the means by which it acts.

Toxicokinetics: The movement of chemicals through the body. This includes rate/extent of absorption, distribution, metabolism and elimination.

TWA-Time Weighted Average: The average exposure concentration over an 8-hour work shift.

Volatility: The ability of a compound to evaporate and partition into the air.

Xenobiotic: Any substance to which an organism is exposed which is not produced internally in that organism at that time.