Toxicology and Potential Health Risk of Chemicals that May Be Encountered by Forest Vegetation Management Workers

PART VI: RISK TO WORKERS USING TRICLOPYR FORMULATIONS (RELEASE® OR GARLON®)

Forest Practices Branch
BC Ministry of Forests

Title Number

8

Abstract

The Release® formulation is the butoxyethyl ester of triclopyr in a kerosene diluent. The Release® product includes 2-butoxyethanol at a concentration of 0.3%. 2-butoxyethanol is commonly used as a solvent in household cleaning substances at concentration of 2–3%. Both substances are of limited toxicity.

Triclopyr is poorly absorbed from the skin, and is excreted by humans and most other species rapidly and without change. The principal effect is skin and eye irritation that may occur after prolonged contact. In experimental animals high oral doses over long periods result in limited and reversible kidney and liver effects. Excretion is through the kidney, largely by a system that can be overloaded if presented with excessive amounts of organic acids, including triclopyr. In that case, concentrations in blood and tissues rise, particularly in the kidney, and the liver processes part of the burden. Triclopyr distribution in tissues and excretion have been evaluated in cattle, goats, rats, rabbits and dogs, and excretion has been investigated in humans. Humans were found to excrete triclopyr very rapidly.

The evidence shows that triclopyr does not have potential to cause cancer or mutation and workers with triclopyr are free of such risks from the herbicide. Reproductive effects occur as delays in development, but only at doses that cause visible maternal toxicity; therefore triclopyr is not considered a reproductive intoxicant. With proper training, discipline and supervision it is very simple to reduce exposure sharply without compromising work efficiency, to raise safety margins.

National Library of Canada Cataloguing in Publication Data

Dost, Frank N.

Toxicology and potential health risk of chemicals that may be encountered by workers using forest vegetation management options. Part VI, Risk to workers using triclopyr formulations (Release or Garlon) Author: Frank N. Dost. Cf. Acknowledgements.

"Title number 8."

Includes bibliographical references: p.

ISBN 0-7726-4987-1

1. Triclopyr – Health aspects. 2. Triclopyr – Toxicology. 3. Herbicides – Toxicology. 4. Weeds – Control – Health aspects. 5. Foresters – Health risk assessment. I. British Columbia. Forest Practices Branch. II. Title.

RC965.F59D67 2003 615.9'02 C2003-960125-0

Acknowledgements

This paper was prepared under contract for Forest
Practices Branch, British Columbia Ministry of
Forests, Victoria, B.C. by Dr. Frank N. Dost, DVM. Dr. Dost is a Fellow of the Academy of Toxicological Sciences, Emeritus Professor of Agricultural Chemistry and Forest Toxicology (Oregon State University, Corvallis), and Affiliate Professor in the Department of Environmental Health (University of Washington, Seattle). The preparation and review of the paper was coordinated by Dr. Jacob O. Boateng, (B.S.F., PhD), Provincial Vegetation Management Specialist, B.C. Ministry of Forests, Victoria.

The assistance of Shona Kelly (formerly Researcher, Health Care and Epidemiology, University of B.C., Vancouver and currently the Lecturer in Social Epidemiology at the University of Nottingham Medical School, Division of Epidemiology and Community Health [U.K.]), Dr. Ray Copes, Medical Specialist (B.C. Ministry of Health, Victoria and Medical Director, Environmental Health, B.C. Centre for Disease Control, Vancouver), Dr. Colin MacKay (Community Medicine Programme, University of B.C., Vancouver), and B.C. Ministry of Forests staff in the review of this paper is acknowledged. Funding for this paper was provided by the B.C.
Ministry of Forests and Forest Renewal British

Columbia (FRBC). Funding assistance by Forest Renewal B.C. does not imply endorsement of any statement or information contained in this publication.

Foreword

Vegetation management is an important reforestation activity for controlling competing vegetation or brush encroachment of young tree seedlings. The activity is necessary to get tree seedlings to free-growing status in most new forest sites established in areas that have been harvested or denuded by wildfire, insects and disease.

There are a number of options for managing forest vegetation. The treatment options include prescribed fire, herbicides, manual removal with hand and power tools (e.g., girdling and slashing tools, chain saws and brush saws), placement of mulch mats, mechanical techniques with heavy machinery, and biological methods. The use of livestock (e.g., sheep) is currently the common biological control technique employed in reforestation areas in British Columbia. Biological methods with insects or specific pathogens is used on forest rangelands for noxious weed control but not commonly used for vegetation control in young forest stands.

The selection of a treatment option involves a decision-making process based on integrated vegetation management concepts that include evaluation of the need for treatment, consideration of all the approved treatment methods and choosing the most appropriate treatment method, monitoring and evaluation. Factors considered in selecting a particular method are the ability of the method to meet the required reforestation objectives, the impact of the treatment at the specific site on human safety and the environment (e.g., recreational resources, fish and wildlife and their habitat, range resources and water supply), as well as the economics of the treatment.

This publication is one of a series of papers that evaluates the potential health effects on forest workers using the commonly employed methods of vegetation control. Other papers in the series are listed at the end of this paper. The emphasis is on risks associated with exposure to chemicals during the use of two most important methods for controlling competing vegetation in regenerated (natural or planted) forest areas. These methods are the use of herbicides and manual removal or control with handheldmotorized (power) equipment.

The herbicides discussed are those that have been commonly used in forestry in Canada. The database on health effects of herbicides is extensive and permits reliable estimates of risk. For components of chain saw exhaust and fuels, there is also voluminous background of toxicological information, but exposure data in forestry is limited. Nonetheless, there is enough information to develop preliminary assessments of potential health effects. While there appears to be a high incidence of physical injury associated with manual methods of brush control, there is virtually no validated data on which to base estimates of risk. The existing data are those of workers compensation boards and insurance companies but such data are generally difficult to obtain or are not specifically enough to characterize the kind of activity that leads to injury.

The information in these reports should provide the basis for important decisions about the way vegetation management in forestry should be carried out, and the use of some forestry activities as a source of assisted employment.

Contents

Introduction

Much of the discussion in this chapter is derived from unpublished detailed summaries of toxicological data submitted in support of registration of triclopyr in the United States and Canada. This information is considered to be proprietary but has been made available to qualified reviewers under agreements of confidentiality. Independently published reports in the open literature are referenced specifically.

The U.S. Forest Service published Background Statements (US Department of Agriculture, 1984) and several Environmental Impact Statements that include thorough discussions of triclopyr as of their time (see US Forest Service, Pacific Southwest Region, 1988; US Forest Service, Southern Region, 1989). In Canada, the regulatory Decision Document for triclopyr was published by Agriculture Canada (1991). A very useful general risk assessment for triclopyr was prepared by Syracuse Environmental Research Associates (SERA)/Syracuse Research Corporation (1996) for the U.S. Forest Service. United States Environmental Protection Agency (USEPA) published its Reregistration Eligibility Decision (RED) document for triclopyr in 1998, reviewing the entire registration database. Both of these documents are extensive and include evaluation of public and ecological impact as well as occupational exposure assessment.

There has been relatively little independent research on the mammalian toxicology of triclopyr because it is of such limited toxicity. However, a number of papers based on the testing required for registration have been published in the refereed literature by staff scientists of the registrant.

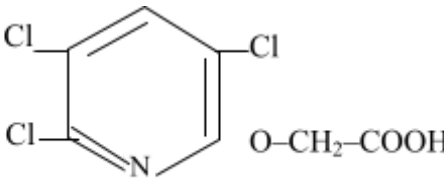
This report is intended as an overview and leaves detailed analyses to the USEPA RED (1998), the Agriculture Canada (1991) Decision Document, and the 1996 risk assessment prepared for the US Forest Service (Syracuse Research Corporation, 1996).

Chemistry

Triclopyr is a herbicide active on broad leaf and woody plants. It is available as the butoxyethyl ester, which is soluble in organic solvents (Release; Garlon 4 in the US) and the triethylamine salt, which is soluble in water (Garlon 3A in the US).

The structural formula for the parent acid is:

The structural formula for the parent acid o



(Molecular formula: $C_7H_4Cl_3NO_3$)

The chemical name is 3,5,6-trichloro-2pyridinyloxyacetic acid. The ester or amine salt replaces the hydrogen of the OH group on the “tail” of the molecule. This is not a strong bond, and when taken into the digestive tract or absorbed into the circulation of animals it separates spontaneously to return to the form shown above.

The structure of triclopyr differs from that of 2,4,5-T only in the presence of a nitrogen atom at the “1” position instead of a carbon. (The conventions of naming chemicals account for the difference in numbering positions because a nitrogen replaced one of the carbon atoms.) However, triclopyr has none of the characteristics that caused removal of 2,4,5-T from use, most importantly the potential for formation of any form of dioxin contaminant during manufacture. It has been alleged that a toxic contaminant similar to a dioxin might form during manufacture. That possibility was anticipated early in the development of triclopyr, and it has been found that such a substance is not

formed in the production of triclopyr. The proposed product was synthesized as an analytical standard and for toxicity testing and was found to have low toxicity and no similarity in biological effect to the dioxins.

Absorption, Distribution, Metabolism and Excretion of Triclopyr

Absorption of triclopyr has been studied in rabbits and human volunteers. Rabbits tend to absorb chemicals across the skin more efficiently than most animals and are a good test species for skin absorption studies. After a single exposure of 2000 mg triclopyr acid/kg body weight applied to either intact or abraded skin of rabbits, the treated area was covered with a bandage to prevent external loss. Average urinary recovery over 24 hours was less than 2% of the applied material, regardless of the condition of the skin. In a subsequent study in rabbits, doses of 125, 250 or 500 mg (acid equivalent) of Garlon 4 formulation (butoxyethyl ester) per kg body weight were applied 5 times weekly for three weeks to the skin. Total recoveries over the three-week period averaged 8-9% for all groups. Dose-related skin irritation was evident in all groups, with severity increasing with dose. The increased absorption noted in the latter experiment may have been due to the kerosene diluent of the formulation, because solvents like kerosene may be expected to cross the lipid-rich skin barrier more easily. The fat-soluble ester itself is also likely to be absorbed more readily than the acid.

Garlon 4 (ester) was applied at a dose of 5 mg/kg to the forearm of 5 human volunteers and left in place for eight hours. The peak blood concentration in the subjects occurred about 12 hours after application. Triclopyr was not detectable in their blood at 72 hours after application. At 96 hours, urinary recovery amounted to 1.37% of the applied material. The amounts recovered from each subject after application to the skin were corrected by the average amount of triclopyr, 80.1%, recovered in urine after an oral dose of 0.5 mg/kg body weight, indicating that an average of 1.65% of the total dose was absorbed (Carmichael et al, 1989). This may be a conservative estimate, because no measure of triclopyr disposition in faeces was attempted, and it is known that retention in tissues is negligible. This estimate is consistent with work by Hotchkiss et al (1992) using full-thickness human skin sections in diffusion cells. They found about 0.7% of radiolabeled triclopyr butoxyethyl ester to have passed through the skin in 72 hours. Application rate was 15 mg/square centimetre.

Species differences in excretion of triclopyr have stimulated a number of studies of its behaviour and excretion. Radiolabeled triclopyr was given to rats as a single intravenous dose of 1, 5, 20, 50 and 100 mg/kg, and an oral dose of 100 mg/kg. Radioactivity was no longer measurable in their blood 30 hours after administration. Sixteen hours after the lower doses about 85% of the material had been excreted, but at a dose of 100 mg/kg excretion was slower. At the lower doses, almost all excretion was by the kidneys. At the high doses a significant amount was excreted in the faeces. This behaviour indicates that the upper limit in the rate at which triclopyr can be excreted by the kidney was exceeded. With the increase in blood concentration, the excess triclopyr was diverted through the liver into the bile. Such a response is consistent with other experiments showing that excretion of organic acids such as triclopyr by the kidney is an active transport process that can be saturated.

Most of the excreted material was unchanged triclopyr, with small amounts of a metabolic product, 3,5,6-trichloro-2-pyridinol, which is triclopyr with the acid side chain removed. In its regulatory decisions on allowable residues, USEPA considers the parent compound and its metabolite together, indicating its conclusion that the metabolite is of similar toxicity. Comparison of triclopyr acid and the butoxyethanol ester showed that they behaved almost identically

when administered orally. This is further evidence that the salts and esters of triclopyr hydrolyse to the parent acid. Findings from studies of the amine, the ester and the parent compound in mammals are therefore interchangeable.

Tissue distribution studies in rats show that 72 hours after single or repeated 3 mg/kg doses, about half of one percent of the administered material was still in the tissues. At a dose of 60 mg/kg, about 2% remained. Data from similar studies with rabbits is consistent with the observations in rats.

Timchalk et al (1990) reported a series of experiments with radiotracer labels to further define the fate of triclopyr in male and female rats. The study included four protocols: single doses of 3 and 60 mg of labelled triclopyr/kg, a loading treatment of 3 mg/kg/day for 14 days followed by a single 3 mg/kg dose of labelled triclopyr, and a fourth group given 3 mg/kg of labelled material as a single intravenous dose. All of the groups provided similar information, with 94-97% of the administered triclopyr recovered, most of which was in the urine. Less than 3% was found in faeces, less than 0.2% was converted to carbon dioxide and less than 2% was found in the tissues 72 hours after administration. The primary difference was a delay in excretion of the high doses due to saturation of the kidney excretory mechanism. In this case, about 9 hours were required for blood levels to decline to a point where the kidney was no longer overloaded. In spite of the difference in dose, kidney performance in terms of percentadministered dose removed was not greatly different. At the end of 12 hours 54% of the high dose had been excreted, compared with 62.5% of the 20 fold lower dose.

A series of excretion studies in dogs was stimulated by the observations that triclopyr excretion was slowed at higher doses in rats, and the well known inefficiency of the dog kidney in excreting organic acids. In one study with dogs, doses of 0.5, 5 and 20 mg triclopyr/kg were given intravenously. Overall recovery was greater than 90%, but as the dose increased, less triclopyr was excreted in the urine and more appeared in the faeces. There was retention in the kidneys and in the bile at the high dose. This is consistent with back-up in the kidney, increased concentration in the circulation, and diversion to the liver. Change to the pyridinol derivative presumably occurs in the liver, where it moves into the bile for delivery to the intestine for excretion.

Monkeys were also evaluated because their excretory processes for such substances parallel that of humans. In the monkey there appeared to be no competition for excretion between triclopyr and other organic acids at the doses tested over an extended period. The animals were given 5 mg/kg/day for 28 days, then 20 mg/kg/day for 102 days. A full range of clinical chemistry tests was conducted periodically, with special attention to tests reflecting kidney function. Even though the high doses caused occasional diarrhoea, weight gain and serum and urine chemistry was normal. There was no effect on clearance of other test organic acids at the highest dose after extended periods of treatment, even when the test material was administered simultaneously with triclopyr. This means that excretory capacity of the monkey kidney was not approached (Timchalk et al, 1997).

The data from the monkey study indicated that there would be no effect on human kidney function, and served as the basis for an investigation of triclopyr absorption and excretion in human volunteers. The doses used were much smaller in humans than in monkeys. Six volunteers were initially given 0.1 mg triclopyr/kg by mouth. After 48 hours, 83.5% of the administered dose had been excreted unchanged in the urine. The second phase of the study utilized a dose of 0.5 mg/kg, of which 80% was excreted. Blood concentrations were proportional to the dose and the time course of excretion was the same for both doses, indicating that kidney saturation was not approached. The slope of the disappearance curve was steep; triclopyr was undetectable 24 hours after

administration. There was no evidence of adverse effects in any of the subjects.

With this baseline data to show efficiency of both uptake from the gut and excretion, Garlon 4 (ester) at a dose of 5 mg/kg was applied to the forearm of five of the volunteers and left in place for eight hours. The peak blood concentration in the subjects occurred about 12

hours after application. Triclopyr was not detectable in their blood at 72 hours after application. The slower loss reflects slower uptake from the skin. At 96 hours after application, urinary recovery amounted to 1.37% of the applied material. The amounts recovered from each subject after application to the skin were corrected by the average amount of triclopyr, 80.1%, recovered in urine 60 hours after an oral dose of 0.5 mg/kg body weight, indicating that an average of 1.65% of the total dose was absorbed (Carmichael et al, 1989). This may be a conservative estimate, because no measure of triclopyr disposition in faeces was attempted, and it is known that retention in tissues is negligible. This estimate is consistent with work by Hotchkiss et al (1992) using fullthickness human skin sections in diffusion cells. They found about 0.7% of radiolabeled triclopyr butoxyethyl ester to have passed through the skin in 72 hours. Application rate was 15 mg/square centimetre.

The information on behaviour of triclopyr is somewhat more extensive than has been necessary for most herbicides, because the initial findings in dogs indicated rather high sensitivity. The reason is the inherent inefficiency of excretion of organic acids by the dog, compared to most other species, particularly humans. Substances like triclopyr and 2,4-D are moved from the central nervous system to the blood stream, and from blood into the urine by an active transport ("pumping") system that has a finite capacity, which is relatively low in the dog. It is the same cellular machinery that disposes of the waste products of neurotransmitter substances from the brain. This relationship is discussed by Timchalk and Nolan (1997). The USEPA acknowledged that for standard-setting for chemicals of this kind the dog is an inappropriate species, which is reflected in the Reregistration Eligibility Decision document (USEPA, 1998) Tissue residues of triclopyr in livestock and game have also been evaluated. Consumption of game from treated areas is not a significant issue compared to occupational exposure, but the behaviour of triclopyr in these animals is added evidence of the rapid clearance of triclopyr from all mammals. Laying hens were given 0.66 mg radiolabeled triclopyr/kg/day by capsule and slaughtered 24 hours after the last dose. Eggs and excreta were collected daily. 82% of the administered material was recovered, 99.7% of which was in excreta. Tissue residues at 24 hours were 0.7 ppm in kidney, 0.2 ppm in blood and less than 0.1 ppm in other tissues, and eggs. Those levels are consistent with the 18% of triclopyr not accounted for, which if uniformly distributed would amount to 0.12 ppm.

In another study, lactating goats were given radiolabeled triclopyr equivalent to 2000 ppm in the feed, a dose of about 60 mg/kg. Browse vegetation soon after treatment can be expected to contain 50 to 300 ppm. Tissue concentration was highest in the kidney, about 8 ppm 24 hours after ingestion of the herbicide. Liver and muscle contained about 0.3 ppm. Milk contained less than 0.2 ppm. The half time for excretion was about 24 hours.

Residues in dairy products were measured by Eckerlin et al (1987). Triclopyr was given in the feed over four days to a Holstein cow at a total dose of 0.7 mg/kg. 84.4% appeared in the urine, none was found in milk or faeces, and at 24 hours after the last dose no further excretion in the urine was detected. This experiment was intended to simulate a 5 ppm dietary intake, which would be a reasonable representation of an area into which drift had occurred, but is substantially lower than concentrations that might be found within a target area. Data from other species indicates, however, that triclopyr ingested at concentrations found in a target zone would be excreted quickly.

The principal metabolite of triclopyr, 3,5,6trichloro-2-pyridinol, constitutes less than 1% of the urinary excretion of triclopyr by humans. Its excretion does not depend on the organic acid transport system, although this has no significance given the very small amount produced.

Acute and Subacute Toxicity

Short-term toxicity of triclopyr is limited; it falls into the category of "slightly toxic" for oral intake or skin contact. Undiluted technical triclopyr is considered to be moderately irritant to the eye. Oral median lethal doses (LD_{50}) of triclopyr acid have been found to be about 700 mg/kg for rats and 300 mg/kg for rabbits. The full formulation of Release or Garlon 4 (61.6% triclopyr butoxyethyl ester) is less acutely toxic than the technical material alone. The LD_{50} of this formulation is in excess of

2000 mg/kg. It is considered non-irritating to the eye. The formulation does cause some skin irritation upon repeated application and occlusion of the treated area. (In this procedure, the area is covered with a patch to hold the material on the skin and to prevent interference by the subject.) It does not cause contact hypersensitivity as judged by standard tests.

The triethylamine formulation (Garlon 3A in the U.S.) has a low acute toxicity similar to that of Garlon 4. It differs in being substantially more irritating to the eye, with detectable persistent injury to the cornea of rabbits several days after application of 0.1 ml of undiluted formulation. Washing of the eye 30 seconds after application did not modify the response. The triethylamine formulation is also moderately irritating to skin of rabbits, although treatment of the ears of rabbits without occluding the area did not result in discernable irritation.

Inhalation is not a significant route of exposure to herbicides, because of the very small volume of respired air relative to the volume of distribution of the herbicide. Exposure of rats for four hours to the highest aerosol concentration achievable (1.84 gm/m^3) was without effect other than eye irritation and a period of lessened weight gain following exposure. Virtually the entire air loading was respirable in the study, compared to a very small fraction of respirable droplets during herbicide application. A similar assay of the Garlon 4 formulation produced a similar response, except that weight gain was not affected.

Dermal toxicity of triclopyr ester is limited. Dilutions of the product were applied to shaved dorsal skin of rats at doses of 54, 540, and 1080 mg of Garlon 4/kg/day, five days a week for three weeks. Each application was washed off after seven hours. The application sites were not covered. The undiluted formulation caused evidence of discomfort and there was dose related skin irritation at all levels. Slower weight gain was seen at all treatment levels. Clinical chemistry and haematology findings included reduction of red cell count and haemoglobin among females at the highest dose rate, marginal reduction in serum proteins in males, and evidence of moderate effects on the liver. Because the treated areas were not covered, there may have been some ingestion of the herbicide, which could have raised intake to levels at which effects have been seen in feeding studies. It is also likely that the prolonged irritation resulting from the exposures exerted secondary effects on liver function.

Subchronic and Chronic Studies

There appears to be some difference in sensitivity to triclopyr between sexes. The standard 13-week oral subchronic study in rats included dose rates up to 100 mg/kg/day and a thorough array of clinical chemistry and pathology analyses. There was little evidence of adverse effect other than a decrease in weight

gain in males at the highest dose, and increased kidney weight. Females showed no evidence of toxicity at the highest dose rate, and males were not affected at 30 mg/kg/day. A 13-week study with a different strain of rats and a maximum dose rate of 250 mg/kg/day showed somewhat similar effects. The maximum dose rate caused marginal liver effects in male rats, which may have resulted from the restricted feed intake, caused by the poor palatability of triclopyr. The highest dose also caused increased kidney weight and decreased body weight gain in both sexes. It is not clear whether palatability played a role in the decreased weight gain. At the next lower dose of 50 mg/kg/day only males were affected. In this experiment, some kidney tubule degeneration was seen at 20 mg/kg/day or more. The responses in the kidney reflect two phenomena. First, the primary route of excretion is by the kidneys, and the concentration of triclopyr in the kidney is higher than in any other organ. Second, the system for excreting organic acids such as triclopyr is a cellular "pump" and has a finite capacity. Over long periods of work at full capacity, cell function begins to fail. At lower doses the system is not working so hard and can function indefinitely. The no-observable-effect level (NOEL) was considered to be 5 mg/kg/day.

The effect of triclopyr on organic acid transport in the kidney is particularly noticeable in the dog, which is less efficient than any other species in this mode of excretion. After 228 days at dose rates of 5, 10, and 20 mg/kg/day in the diet, beagles showed some evidence of kidney and liver effects, even at the lowest level. (Higher doses were not palatable.) A second study at doses of 0.1, 0.5 and 2.5 mg/kg/day was directed at understanding the observations of the previous study and finding a NOEL. One test used was a standard kidney function assay with a dye that is excreted by the same mechanism as that which excretes triclopyr. Triclopyr interfered with excretion of the dye at a dose rate of 2.5 mg/kg/day. There was no accompanying evidence of toxicity or pathological change and the competition for excretion was not considered to be a toxic response. The NOEL was determined to be 2.5 mg/kg/day.

A further study of dogs over a full year was done to meet more recent regulatory domestic and overseas registration guidelines. Dose rates were 0.5, 2.5 and 5 mg/kg/day. The work disclosed little additional information, again providing a NOEL of 2.5 mg/kg/day. Animals in the highest dose range also showed moderate liver effects. One difficulty in conducting these tests stems from the bad taste of triclopyr, which apparently contributed to lowered feed intake and weight loss.

Chronic toxicity evaluation was carried out simultaneously with the most recent carcinogenicity assays for triclopyr and included extensive evaluation of haematology, blood chemistry and urinary constituents. In rats, at dose rates of 3, 12, and 36 mg triclopyr/kg/day, the only findings were dose-related increases in pigmentation within the kidneys of females, and some degeneration of kidney tubules in males. The most recent mouse study included dose rates of approximately 6, 31, and 156 mg/kg/day.

Clinical chemistry and haematological findings were unremarkable. Weight gain at the highest dose was below normal, and an increased incidence of thymus enlargement occurred in males at the two highest doses.

Reproductive Effects, Including Fertility, Developmental Effects and Foetal Loss

In a three-generation reproduction assay, groups of males and females were administered triclopyr in the diet at doses of 0, 3, 10 and 30 mg/kg/day, beginning eight weeks prior to the first mating. In this test, both sexes are fed the chemical over a period long enough prior to mating to affect all stages of the spermatic generation cycle. The diet is continued through gestation and until weaning

of the offspring. The offspring are then maintained on the diet through their full reproductive cycle, as are their offspring. All aspects of reproductive performance and health of offspring are monitored.

No effects on reproductive capacity by triclopyr were found. The study was slightly impaired by a decrease in the number of females that mated compared with the number paired with males. This occurred at all doses and in the controls and appeared to have no relation to the compound. The number of pregnancies per mating was not affected. No changes in litter size, survival or other indices were evident. Kidney pathology was specifically studied because of findings discussed earlier, but no effects were found. The no-effect level was 30 mg/kg/day.

Two series of rabbit teratogenicity assays were conducted. The first was apparently negative, but an enteritis outbreak in the colony resulted in questions about the validity of the information. In the second series, doses of 10, 25 and 75 mg triclopyr/kg/day were given to pregnant rabbits from day 6 to day 18 of gestation, and the foetuses were examined on the 28th day. There was no increase in foetal defects at any dose, even though there was significant maternal toxicity at the highest dose.

The dose rates for the teratogenicity assay in rats were 50, 100, and 200 mg/kg/day. Weight gain was slightly impaired at 100 and 200 mg/kg/day, and all treated groups showed some signs of maternal toxicity, varying with the dose rate. Body weights of pups born to females treated at 200 mg/kg/day were slightly decreased, and there was evidence of foetal toxicity in the form of delayed bone development. Of the 277 foetuses from high dose females, two had major structural defects, an incidence comparable to that in untreated rats in other studies. There was no effect at a dose rate of 100 mg/kg/day. The no-effect level for maternal toxicity was 50 mg/kg/day, based on the slight weight loss, and 100 mg/kg/day for foetal effects.

The results from these assays indicate that triclopyr has no potential for inducing miscarriage, reproductive failure or birth defects at doses that do not cause other serious toxicity.

Mutagenic Effects (Genetic Damage)

Triclopyr has been assessed for mutagenic activity with an extensive battery of assays. It was inactive in five tester strains of Salmonella in the Ames assay. A host-mediated assay, in which mice were treated with triclopyr at doses up to the tolerable limit, then injected with mutation-sensitive test bacteria, produced no response, while known mutagens were positive. A bone marrow cytogenetic assay for chromosomal aberrations was negative at doses up to the maximum of 70 mg/kg. The dominant lethal assay, which responds to male germ cell mutation, was also negative. Testing for unscheduled deoxyribonucleic acid (DNA) synthesis (UDS) was negative. UDS occurs when DNA damage is greater than background, requiring greater than normal repair activity. A negative finding indicates no unusual DNA damage.

The evidence indicates that triclopyr does not cause genetic injury or mutation at the level of DNA, the chromosome or the whole cell.

Carcinogenicity

The ability of triclopyr to induce cancer has been evaluated in rats and mice. The first study in rats was conducted by Industrial Biotest Laboratories, which was later found to have done defective work. Although the data was validated as showing no response, it was considered insufficient to make an adequate judgement of the carcinogenic potential of the compound.

The work was repeated with daily intakes of 3, 12, or 36 mg triclopyr/kg/day, with 50 animals of

each sex per dose rate. Additional animals were included for interim observations. There was a small increase in mammary adenomas (benign tumours) and in mammary adenocarcinomas in the high dose females, compared to control animals. Neither effect was significant alone, but when combined they were significant. The control animals in the study had an unusually low number of these tumours compared with historical controls, which led to the apparent statistical increase in the treated animals. The incidence in the treated groups was not different from the historical incidence in rats of that strain. Other information suggests that the finding is an anomaly. The findings were reviewed to address Canadian regulatory concerns, with the conclusion that observed tumours were not related to triclopyr administration. There were no animals with multiple tumours, no tumours in males, no evidence of increasing incidence with time of exposure, and no dose response.

In addition triclopyr does not have significant mutagenic activity, and is excreted rapidly and unchanged, which further diminishes the probability that the observed difference represents a real effect.

The mouse study employed feed concentration of 0, 24, 80, and 240 parts per million, corresponding to dose rates of approximately 0, 3, 10, and 30 mg/kg/day. The strain of mouse used has a history of urinary tract disease, which led to termination of the males at 97 weeks. However, this was a long enough period for adequate study results. The females were not as seriously affected and completed the planned 104 week treatment. Curiously, survival was highest in the high dose group.

There was an apparent increase in benign adenomas in the lungs of treated males and females when compared to the control group assigned to this study. However, the incidence of benign lung adenomas in the treated group was not significantly different from another control group in the same facility at the same time under identical conditions. There was no dose response relationship across the ten-fold range of treatments, and the tumours found were solitary and considered characteristic of spontaneous rather than induced tumours. The strain of mouse used is noted for highly variable incidence of lung tumours. Due to questions raised by the original mouse observations, particularly because of the normally high incidence of kidney and lung involvement of the strain that was used, another assay was conducted and the results reported in 1987. Dose rates in the latter study were considerably higher, at dietary concentrations of 50, 250, and 1250 parts per million, which is equivalent to dose rates of about 6, 31, and 156 mg/kg/day. Larger numbers of animals were employed; there were 60 animals per sex per dose for the full term of 22 months, plus 40 animals per sex per dose for evaluation during the course of the study. A full range of clinical chemistry and blood characteristics was observed during the study. There was no evidence for a carcinogenic effect.

The US Environmental Protection Agency has concluded that the rat and mouse studies showed, in each case, that there were "--no carcinogenic effects observed under the conditions of the study." (Federal Register 60:4093-4095, Jan 20, 1995) Nonetheless EPA has identified triclopyr as Group D, "not classifiable as to human carcinogenicity" in spite of the stated evidence. EPA has concluded that a cancer risk assessment for triclopyr is not necessary. (Federal Register 62:46888-46894, September 5, 1997)

The classification in Group D is a curious position. EPA has concluded that all necessary studies have been conducted and that no carcinogenic effects were observed. A six member Pathology Working Group commissioned according to USEPA regulation reviewed all four carcinogenicity studies and concluded that the overall weight of the evidence indicates that triclopyr is not carcinogenic in either rats or mice. Apparently the Working Group recommendations and its own findings are not incorporated in the EPA conclusion.

Risks to Workers Associated With Use of Triclopyr Ester

In studies described in the assessment by Syracuse Environmental Research Associates for the US Forest Service (1995) the maximum estimated intake by workers applying triclopyr ester by backpack was 0.006 mg/kg per pound (0.454 kg) of active ingredient applied.

Four to six pounds (1.82 to 2.72 kg) of triclopyr were applied per day, providing absorbed doses of 0.024-0.036 mg/kg/day. These numbers can be related to oral studies in animals because of efficient absorption from the digestive tract.

In animal experiments over long periods, the noobserved-effect level (NOEL) established by USEPA is 5 mg/kg/day and the reference dose (RfD) is 0.05 mg/kg/day. The RfD is the lifetime daily intake considered to be without potential for adverse effect.

The margin of safety for workers relative to the NOEL is 166, or 1.66 relative to the reference dose. However, a careless worker may have higher exposures, bringing the margin of safety down, but even a doubling of exposure leaves a substantial safety factor. With proper training, discipline and supervision it is very simple to reduce exposure sharply without compromising work efficiency, to raise safety margins.

Triclopyr is not a carcinogen or mutagen, and workers using triclopyr are free of such risks from the herbicide. The most important concern to workers is skin and eye irritation, which can be avoided with careful work habits.

References

Agriculture Canada, Food Production and Inspection Branch, Pesticides Directorate (1991). Decision Document E91-02, Triclopyr. Available from Health Canada, Pest Management Regulatory Agency, at www.hc-sc.gc.ca/pmra-arla/

Borrecco, J.E. and Neisess, J. (1991) Risk assessment for the impurities 2butoxyethanol and 1,4-dioxane found in Garlon 4 and Roundup herbicide formulations. Report no. R91-2, Forest Pest Management, Pacific Southwest Region, USDA Forest Service.

Carmichael, N.G., Nolan, R.J., Perkins, J.M., Davies, R. and Warrington, S.J. (1989) Oral and dermal pharmacokinetics in human volunteers. Human Tox. 8:431-437.

Eckerlin, R.H., Ebel, J.G., Maylin, G.A., Muscato, T.V., Gutenmann, W.H., Bache, C.A. and Lisk, D.J. (1987) Excretion of triclopyr herbicide in the bovine. Bull. Envir. Contam. Toxicol. 39:443-447.

Ghanayem, B.I., Burka, L.T., Sanders, J.M. and Matthews, H.B. (1987) Metabolism and disposition of ethylene glycol monobutyl ether (2-butoxyethanol) in rats. Drug Metab. and Dispos. 15:478-484.

Hotchkiss, S.A.M., Hewitt, P., Caldwell, J., Chen, W.L. and Rowe, R.R. (1992) Percutaneous absorrption of nicotinic acid, phenol, benzoic acid and triclopyr butoxyethyl ester through rat and human skin *in vitro*: Further validation of an *in vitro* model by comparison with *in vivo* data. Food Chem. Toxicol.30:891-899.

Syracuse Environmental Research Corporation (SERA)/Syracuse Research Corporation (1996) SERA TR 95-22-02-02a Selected commercial formulations of triclopyr-Garlon 3A and Garlon 4. Risk assessment. Final report to US Forest Service.

Timchalk, C., Dryzga, M. D. and Kastl, P. E.(1990) Pharmacokinetics and metabolism of triclopyr (3,5,6-trichloro-2pyridinyloxyacetic acid) in Fischer 344 rats. Toxicology 62:71-87.

Timchalk, C., Finco, D.R. and Quast, J.F. (1997) Evaluation of renal function in rhesus monkeys and Comparison to Beagle Dogs

Following Oral Administration of the Organic Acid Triclopyr (3,5,6-Trichloro-2pyridinyloxyacetic Acid. Fund. Appl. Toxicol. 36:47-53.

Timchalk, C. and Nolan, R.J. (1997) Pharmacokinetics of Triclopyr(3,5,6Trichloro-2-pyridinyloxyaceti c Acid) in the Beagle Dog and Rhesus Monkey: Perspective on the Reduced Capacity of Dogs to Excrerte this Organic Acid Relative to the Rat, Monkey and Human. Toxicol. Appl. Pharmacol. 144:268-278.

US Department of Agriculture (1984) Pesticide Background Statements, Volume I: Herbicides. Agriculture Handbook No. 633. U.S. Govt. Printing Office, Washington, D.C.

US Forest Service, Pacific Southwest Region. (1988) Final Environmental Impact Statement. Vegetation Management for Reforestation. Volume III.

US Forest Service, Southern Region. (1989) Final Environmental Impact Statement. Vegetation Management in the Appalachian Mountains.

USEPA (1998) EPA 738-R-98-011 Reregistration Eligibility Decision (RED) Triclopyr.

WCB (1999) Occupational Health and Safety Regulations. Workers Compensation Board of British Columbia. Richmond, B.C.

Glossary

Active Transport – Molecules within cells or in fluids outside the cells may move across membranes passively by diffusion**,** just as a drop of dye might spread in water, or by an energy consuming process where the molecule is moved across a membrane into another compartment where the concentration may be higher. Without this "pumping" mechanism molecules would move the wrong way. The best examples of this active transport process with respect to herbicides is the movement of organic acids like 2,4-D and triclopyr from the brain to the blood and from the blood into the kidney tubules for excretion. Glyphosate, on the other hand, moves out by diffusion without help from active transport.

Acute toxicity – (Short term toxicity) –Acute toxicity is the quality or potential of a substance to cause injury or illness from a single dose or short period of exposure. See **subacute, subchronic and chronic. Adjuvant –** Any additive to a pesticide formulation that is not active itself, but is intended make the active ingredient work better.

Cancer – A malignant growth of potentially unlimited size that invades local tissues, and may spread to other parts of the body.

Carcinogen – A chemical capable of inducing cancer.

Carcinogenic – Capable of causing cancer.

Chronic toxicity – (Long-term toxicity) – Chronic toxicity is the quality or potential of A substance to cause injury or illness after repeated exposure for a long period of time. Chronic toxicity tests run for a year or more; for rodents the period may extend through the entire life span. A chronic effect persists for months or years and may arise from acute or long term exposure. See **acute, subacute, subchronic.**

Contaminant – In a formulation, usually residues or impurities from the manufacturing process present in small quantities. Contaminants must be identified to the regulatory agency, which judges whether they are of concern.

Deoxyribonucleic Acid – See **DNA.**

Dose – The amount of a chemical that actually enters the body to be distributed to all of the organs and cells. Distribution to tissues and cells is selective, and depends on the nature of the chemical and characteristics of each kind of cell.

Dose-response relationship – The central idea in toxicology and in pharmacology (which is the science dealing with beneficial effects of therapeutic drugs). As the dose (or

concentration) of a chemical increases, the effect increases, and as the dose is lowered, the effect becomes less. This response pattern applies to every interaction between a chemical and a biological system, whether human, fish, bacteria or any other kind of organism or tissue. The dose-response relationship is absolutely essential to judgement of the effect of any chemical.

DNA (Deoxyribonucleic Acid) – The genetic library in each cell that contains all of the instructions for building and operating the body. Each kind of cell contains all of the information for the whole body. Only the information needed for each kind of cell is used by that cell; the rest is repressed. Liver cells do not try to be muscles, and muscles do not try to become brain cells, but they contain all of the information**.**

Epidemiology – The scientific study of the cause, distribution, and control of epidemics or other disease in a region. In the context of these reports, epidemiology is the study of possible associations between environmental and occupational chemicals and occurrence of diseases. The term "associations" is used in its statistical sense, which means that the relationship cannot demonstrate cause and effect.

Exposure – Amount of a chemical that reaches a surface from which it might be absorbed. The dose is some fraction of the exposure. Exposure does not include material that is on nearby foliage or other surfaces. It is only the material that reaches the skin (by contact), respiratory tract (by inhalation) or digestive tract (by ingestion).

Foetus – The later stage of mammalian development in the womb. In human, this refers to the unborn child during the period of uterine life from the end of the second month until birth.

Formulation – A complete pesticide preparation as sold by a manufacturer for practical use. It includes the active ingredient and any necessary adjuvants and solvents. For use, it may or may not require further dilution or mixing with other substances. Formulation can also be defined as the process used by manufacturers in preparing a pesticide for practical use.

Half-life – The length of time required for disappearance of half of the material present in an organism or in environmental media. It is a more useful idea than "persistence" because it allows prediction of the time required to reach low target levels with out making measurements over exceedingly long periods. A better term is "Half-time," because the information only relates to a given location, and says nothing about the processes that deplete the chemical. If it evaporates or is carried away intact by water it may still exist in its original form. The term "half-life" originated with description of radioactive decay, in which elements become a totally different substance. The English language sometimes loses precision as it evolves.

Herbicide – A chemical substance or cultured biological organism, used to kill or suppress the growth of plants.

Immune system – All of the structures and cells and their products that protect against infectious organisms and against cells of the body that have become altered in the very early development of cancer.

Inert ingredient – Any component of a formulation that is purposely added and does not have pesticidal activity. Includes solvents and adjuvants, not manufacturing impurities.

Irritation – A purely local or topical reaction which may include redness, blistering, swelling, burning or itching.

Lethal – Causing death.

LD_{50} – Acronymn for Median lethal dose.

Lethal concentration (LC_{50}) – Rate at which 50 percent of test animals will be killed. **LOAEL** – Acronym for lowest-observedadverse-effect level.

Lowest-observed-adverse-effect level (LOAEL) – The lowest measured amount of a chemical that produces significant increases in frequency or severity of adverse effects in exposed subjects. in the general sense it includes all biochemical, pathological, behavioral, reproductive, genetic and other measurable changes. the term may also be applied to any specific parameter under observation.

Malignant – Deadly or very injurious. As applied to cancer, invasive of local tissues and metastatic (migration of cancer cells to other tissues).

Margin of Safety (MOS) – The difference between the estimated dose of a pesticide and the NOAEL. A **MOS** of 100 (estimated dose 100 fold less than the NOAEL) is usually considered to assure that no adverse effects will occur.

Median lethal dose (LD_{50}) – The dose of a chemical, biological agent, or other substances that causes death in 50% of defined test animals.

Metabolism – The sum total of the biochemical reactions that a chemical undergoes in an organism. The processes include biochemical (enzymatic) reactions in the cells of the body that convert nutrients to energy and structural materials of the body; reactions that change wastes so they can be removed; and reactions that convert foreign substances, such as some pesticides to forms that can be excreted.

MOS – Acronym for margin of safety.

Mutagenic – Capable of producing genetic changes.

Mutagens – Chemicals that are able to induce gene or chromosome damage that is stable and survives cell division to reach the next generation of cells. See **mutation.**

Mutation – Genetic change in DNA of a cell that can be transmitted to the next generation of cells. If in sperm or egg cells, a mutation may be transmitted to offspring. If in somatic (body) cells such as liver, muscle or other organs, a mutation may pass to daughter cells in the organ. The change may have no effect on cell function or it may damage the cell, or even imaginably improve it.

NOAEL – Acronym for **no-observed-adverseeffect level.**

No-observed-adverse-effect level (NOAEL) – The dose rate or concentration at and below which no adverse effects can be detected. (See **threshold; SEE LOAEL)** If the estimated dose of a herbicide to a worker is very low compared to the **NOAEL** for the most sensitive effect found in the laboratory, no harmful effect is to be expected.

NOEL – Acronymn for **no-observed-effect level.**

No-observed-effect-level – (NOEL)-Dose of a chemical or biological agent at which there are no biologically or statistically significant effects attributable to treatment. The term can refer to adverse, beneficial or meaningless effects and is falling out of use in toxicology.

Persistence – The duration of measurable concentrations of a pesticide in soil, foliage or other media. (See Half-life.)

Pesticide – Any chemical (or biological product) intended to control or kill pests. Herbicides, insecticides, fungicides are all pesticides. The term is sometimes incorrectly used to mean only insecticide, for example "pesticides and herbicides." **Pharmacokinetic** – Relating to the rate and pattern of the absorption, distribution, metabolism and excretion of drugs in an animal.

RfD – Acronym for reference dose.

Reference dose (RfD) – Any oral dose below the RfD is considered unlikely to be associated with an adverse health effect and is therefore acceptable. The RfD is usually based on the most sensitive oral NOAEL, with all appropriate safety factors included. **Registration** – The process by which government (e.g., Canadian federal government) authorities determine that a pesticide is suitable for use. Standards of public

and worker safety, environmental impact, and usefulness must all be met.

Risk – The probability (likelihood) that some adverse or undesirable effect will take place in the future, as a result of some specified activity. Risk may relate to health, finances or any other kind of undesirable impact. Real risk may be so small that it cannot be distinguished from zero, or so great that it is a certainty. In the context of pesticides, risk is the probability that use of the pesticide will result in some specified harmful effect on workers or the public. Risk assessment is the process of estimating that probability.

Safety Factor – See **Margin of Safety.**

Subacute – Extending over a few days to perhaps a month. This and related terms do not carry defined time periods; consequently there is overlap in the way they are used. See **Acute, subchronic and chronic. Subchronic –** For experimental studies, relatively long term, but not as long as a chronic study. Typically three to six months. See **acute, subacute, and chronic.**

Teratogen – A chemical that can cause birth defects.

Teratogenic – Relating to or able to produce birth defects.

Threshold – The lowest dose that will produce a given effect. As a practical matter, the threshold is little different from the **NOAEL.**

Tolerance – Lesser than normal sensitivity of an individual to the adverse effect of a chemical. also, the allowable residue of a pesticide on a food or feed crop.

Toxicant – A toxic agent; a poison.

Toxicity – The whole pattern of harmful effects (illness and other undesirable effects) that a chemical can cause. It is a property of the chemical; it does not change.

Toxicology – The group of scientific disciplines that identifies and studies the adverse effects of chemicals on biological systems, whether in the laboratory or in the field.

Toxin – A poisonous substance produced by a living organism. The term is sometimes incorrectly used in reference to nonbiological chemicals.

APPENDIX I: Inert ingredients Triclopyr formul

Inert ingredients are materials without pesticidal activity added to the formulation as diluents, preservatives, and surfactants and for other purposes necessary to effective storage or use of the formulation. While inert ingredients are inert with respect to the function of the active ingredient, some may have considerable toxicity. It is only recently that regulators have begun examining inert ingredients for potential harmful activity. There are pesticide formulations not used in forestry that contain such materials as formaldehyde or benzene, which are significant toxicants. In forestry, most inert materials are diluents such as kerosene or diesel fuel, or surfactants, which are detergents similar to those used in households, or substances intended to prevent formation of very small droplets. Contaminants are usually substances left over from the manufacturing process and have no intended function in the formulation.

In triclopyr ester formulations the inert ingredient of concern is kerosene, which is present as a solvent. A risk assessment for kerosene was prepared by the U.S. Forest Service, Pacific Southwest Region (1988).

Kerosene is classified as very slightly toxic; the median lethal dose is 28,000 mg/kg. It is a skin irritant, and is apparently not mutagenic or carcinogenic.

A manufacturing impurity, 2-butoxyethanol (also known as ethylene glycol monobutyl ethanol, (EGBE)) may be present in Garlon 4 at a concentration of about 0.3%. It is also a widely used industrial solvent and is a component of many household cleaning products, where concentrations average slightly less than three percent (Ghanayem et al, 1987). Borrecco and Neisess (1991) prepared a risk assessment for 2butoxy ethanol. No-effect doses for all major indices of toxicity are similar or higher than those for triclopyr ester itself. If its concentration is a very small fraction of that of triclopyr itself, and its toxicity in various indices is less than that of triclopyr, it cannot contribute significantly to the toxicity of the formulation. The permissible eight-hour exposure limit for workers is 25 ppm (120 mg/m^3) (WCB, 1998).

Titles in this Series

1 Principles of health effects evaluation and risk estimation for chemicals that may be encountered in forest vegetation management

2 Pesticide testing for registration: toxicity, environmental behaviour, and epidemiology

3 Toxicology and potential health risk of chemicals that may be encountered by workers using forest vegetation management options. Part I: Risk to workers associated with exposure to emissions from power saws

4 Toxicology and potential health risk of chemicals that may be encountered by workers using forest vegetation management options. Part II: Exposure to and absorption of herbicides used in forestry

5 Toxicology and potential health risk of chemicals that may be encountered by workers using forest vegetation management options. Part III: Risk to workers using 2,4-D formulations

6 Toxicology and potential health risk of chemicals that may be encountered by workers using forest vegetation management options. Part IV: Risk to workers using glyphosate formulations (e.g., Vision , Roundup , Vantage Forestry and Forza)

7 Toxicology and potential health risk of chemicals that may be encountered by workers using forest vegetation management options. Part V: Risk to workers using hexazinone formulations (Pronone , Velpar L)

8 Toxicology and potential health risk of chemicals that may be encountered by forest vegetation management workers. Part VI: Risk to workers using triclopyr formulations (Release , or Garlon 4)

9 Toxicology and potential health risk of chemicals that may be encountered by workers using forest vegetation management options: Summary