

EFFECTS OF CHLORFENAPYR ON ADULT BIRDS



Peter H. Albers (USGS), Mark J. Melancon (USGS), David E. Green (USGS), Patrice N. Klein (FDA), Brian Bradley (UM Baltimore Co.), David J. Hoffman (USGS), George Noguchi (FWS)



INTRODUCTION

Chlorfenapyr is the first commercial pesticide to be derived from a class of microbially-produced compounds known as halogenated pyroles. Synthesized in 1988 from a naturally-produced chlorinated pyrrole, chlorfenapyr (AC 303,630 Technical: 4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile) is being used in more than 30 countries, including the United States. Chlorfenapyr is a 'proinsecticide', i.e., it requires activation through metabolism. The parent compound is converted to a metabolite, which functions as an uncoupler of oxidative phosphorylation at mitochondria. The primary, and most toxic, metabolite is the N-dealkylated compound AC 303,268 (Fig. 1). Chlorfenapyr has low volatility and water solubility; is lipophilic; binds strongly to soil particles; and degrades slowly in soil (avg. half life of 1 yr), sediment (avg. half life of 1.1 yr), and water (avg. half life of 0.8 yr). Biological evidence presented by the manufacturer (BASF) indicates that chlorfenapyr is rapidly metabolized and excreted by mammals, birds, and fish; hence, unlikely to bioaccumulate in individual organisms or biomagnify between trophic levels.

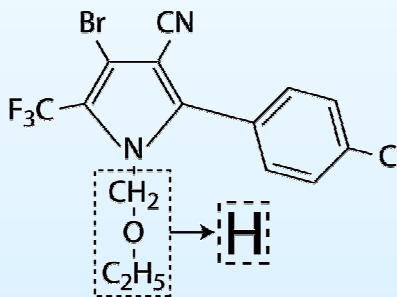


Figure 1. Molecular structure of chlorfenapyr showing the result of metabolism.

Emergency exemptions to use chlorfenapyr on cotton have been granted by EPA for at least 11 states since 1995. In March 2000, the registrant withdrew its application for use on cotton in the U.S. because of a very negative review by EPA. Critical scientific input from government and non-government scientists and environmental groups, supported an EPA risk assessment that revealed excessive risk to wildlife; the results of laboratory avian toxicity tests were central to the EPA argument. In January of 2001, technical chlorfenapyr was unconditionally registered with EPA as a pesticide and a formulated product (PYLON) was registered as a miticide for use in commercial greenhouses. In December 2001, a formulated product (PHANTOM) was conditionally registered as a termiticide.

Chlorfenapyr has high acute, sub-acute, and chronic (reproductive) toxicity in birds; and poses an acute poisoning hazard to aquatic organisms (definitive data are lacking for chronic effects). Results of experiments with laboratory mice and rats revealed a number of histological abnormalities, including vacuolation of brain, spinal cord, and optic nerve tissue in mice and vacuolation of spinal nerves and myelin sheath swelling in rats. As a consequence, the EPA health (human) effects risk characterization contains a recommendation for a developmental neurotoxicity study. Because the registrant is expected to again try to register chlorfenapyr for use on cotton and other outdoor uses, it is important to increase our knowledge of the biological effects of chlorfenapyr on wildlife.

OBJECTIVES

1. Describe the pathological effects (macro and micro) of chronic exposure to chlorfenapyr.
2. Identify biochemical responses that can be used to diagnose exposure to chlorfenapyr.
3. Determine tissue retention potentials for parent compound and primary metabolite.

METHODS

Adult mallards were fed diets containing technical or formulated chlorfenapyr in concentrations that cause effects ranging from a moderate incidence of death to no deaths. Feeding trials were performed in 2002 and 2003 beginning in early July and ending in mid-September. Mallards that died during the study and mallards that were euthanized during the study or at study termination were necropsied and tissues were removed for histological examination, biochemical evaluation, and chemical analysis for chlorfenapyr and the primary metabolite.

The 2002 study consisted of 55 adult mallards purchased from a commercial source. Ducks were in elevated outdoor pens, one bird per pen. Each pen had a food container, flowing water, and shade. Ducks received diets containing chlorfenapyr mixed into pelleted duck food (Fig. 2). Mallards received diets containing either 0 ppm (15 ducks), 2 ppm (10), 5 ppm (10), or 10 ppm (10) technical chlorfenapyr; or 5 ppm formulated chlorfenapyr (10). Samples of mixed diet for all nominal concentrations were saved for chemical analysis. Food consumption was estimated for weeks 1, 2, 3, 5, 7, and 9 by subtracting feed left in the food container from the food added (Fig. 3).



Figure 2. Chlorfenapyr being mixed into the diet.



Figure 3. Food containers being weighed and feed being added.

Mallards were weighed at study onset and at weekly intervals thereafter. Necropsies were performed within 24 hrs of death (Fig. 4). We removed the brain, sciatic nerve, spinal cord (neck and lumbar), skeletal muscle, heart, proventriculus, duodenum, liver, kidney, pancreas, and thyroid for histological examination. We also removed the ventriculus (gizzard), testes, lung, spleen, thymus, jejunum, ileum, cloaca, and adrenal for possible histological use. A portion of the liver was frozen and saved for chemical analysis for chlorfenapyr and the primary metabolite. At study termination, all ducks were euthanized and processed, as were birds that died during the study. A subset of the ducks was tested for the presence of West Nile virus antibodies.



Figure 4. Necropsy of a mallard that died during the study.

Biomarkers have not been identified for the exposure of wildlife to chlorfenapyr. Because most toxic agents impact proteins, such as enzymes, examination of the suite of proteins present in a tissue or an organism is an attractive approach for identifying a biomarker for chlorfenapyr. Such protein studies are part of the field called proteomics. We are using this as a very broad approach to look for changes in the protein composition in selected tissues from chlorfenapyr-treated animals as compared to controls. Basically, this involves subjecting tissue protein extracts to two-dimensional polyacrylamide gel electrophoresis, followed by visualization and use of a computer program to compare the resulting protein patterns from treated and control animals. Protein patterns could be used as biomarkers or they might provide leads for specific proteins, such as enzymes, that could serve as biomarkers by direct assay. Blood and liver tissue from a subset of the ducks were used for the development of methods for protein pattern analysis.

The 2003 study consisted of 80 adult mallards divided into two groups. One group of 60 ducks received diets containing either 0 ppm (20 ducks), 5 ppm (20), or 10 ppm (20) technical chlorfenapyr for 5 weeks, followed by 5 weeks on 0 ppm for all ducks. Two ducks from each group were euthanized on days 3, 7, 22, 37, and 49 of the study. Tissues for histological examination and chemical analysis were saved as in the 2002 study. Blood, liver, and kidney samples were saved from ducks euthanized during the study, plus two ducks from each group during the terminal necropsy, for biochemical assays. Food consumption and body weight were measured and diet samples saved as in the 2002 study. A second group of 20 ducks received diets containing either 0 ppm (10 ducks) or 5 ppm (10) technical chlorfenapyr for 3 weeks. Blood was collected prior to receiving treated diets, after 1 day on diet, and after 10 days on diet for a protein pattern analysis. Eight of the ducks were euthanized on day 17 and used for an assessment of protein patterns during a 48-hour period after death. Fresh blood or serous body fluid and portions of brain tissue, spleen, and kidney were collected from all ducks for West Nile virus testing.

RESULTS

Data are undergoing statistical evaluation. Microscopic evaluation of tissues, protein array analysis, biochemical analysis, chemical analysis, and West Nile virus determinations are incomplete.