



Available online at www.sciencedirect.com



Veterinary Parasitology xxx (2007) xxx–xxx

veterinary
parasitology

www.elsevier.com/locate/vetpar

Toxicological properties of metaflumizone

K. Hempel^a, F.G. Hess^b, C. Bögi^a, E. Fabian^a, J. Hellwig^a, I. Fegert^{a,*}

^a BASF Aktiengesellschaft, GUP/CP and GV/T-Z470, D-67056 Ludwigshafen, Germany

^b BASF Corporation, Research Triangle Park, NC 27709, USA

Abstract

Metaflumizone is a new insecticide developed for crop protection and urban pest control by BASF. Its mammalian toxicological profile was assessed by conducting multiple toxicity studies in the rat, mouse, and dog, covering all relevant endpoints. Metaflumizone is characterized by very low acute toxicity, is not irritating to the eye or the skin and does not possess a potential to induce skin sensitization. The substance also shows relatively low toxicity following subchronic oral or dermal exposure to mammals. In addition, metaflumizone demonstrates low toxicological potential following chronic oral exposure to rats, mice, and dogs. Overall, the lowest no observed adverse effect level (NOAEL) is 12 mg/(kg day) from the 1-year chronic dog study. In a battery of *in vitro* and *in vivo* mutagenicity assays, the weight-of-the-evidence indicates a lack of potential genotoxicity for metaflumizone. Furthermore, the compound demonstrated a lack of potential oncogenicity in long-term toxicity studies in rats and mice. Results from the rat multi-generation reproductive toxicity study as well as the rat and rabbit developmental toxicity studies indicate that metaflumizone is not selectively toxic to the offspring or fetus, as compared to the parents. Also, metaflumizone is not teratogenic in the rat or rabbit. Lastly, no neurotoxicity could be detected in acute and subchronic neurotoxicity studies in rats.

© 2007 Elsevier B.V. All rights reserved.

Keywords: Metaflumizone; Insecticide; Mammalian toxicology

1. Introduction

Metaflumizone is a new insecticide belonging to the semicarbazone class of chemistry. It offers a combination of high efficacy against key agriculturally and medically important insect pests and low risk to non-target organisms including beneficial insects and pollinators as well as humans and the environment.

A complete data package on the toxicological properties of metaflumizone, conducted in accordance with internationally accepted guidelines has been completed and is presented below.

2. Testing

All the studies conducted to identify the toxicological profile of metaflumizone were performed according to Good Laboratory Practices (GLP) at laboratories following internationally accepted test guidelines. The test battery is detailed in Table 1. In most cases, metaflumizone (CAS No.: 139968-49-3, IUPAC: (EZ)-2'-[2-(4-cyanophenyl)-1-(a,a,a-trifluoro-*m*-tolyl)ethylidene]-4-(trifluoromethoxy)carbanilohydrazide) was tested as technical material; for absorption, distribution, metabolism and excretion studies metaflumizone was radio-labeled with Carbon¹⁴ (¹⁴C) in either the benzoni-trile-ring (B-label) or in the trifluoromethoxyphenyl-ring (T-label) of the molecule. When formulated material was evaluated this was a suspension concentrate formulation. For oral administration in rodents metaflumizone was suspended in 0.5% carboxymethylcellulose (CMC) and

* Corresponding author. Tel.: +49 621 60 58133;
fax: +49 621 60 58134.

E-mail address: ivana.fegert@basf.com (I. Fegert).

Table 1
Listing of the toxicological studies and the test guidelines of conducted studies with metaflumizone

Study	Guideline
Toxicokinetics	OECD 417
Acute oral toxicity	OECD 401
Acute dermal toxicity	OECD 402
Acute inhalation toxicity	OECD 403
Acute dermal irritation/corrosion	OECD 404
Acute eye irritation/corrosion	OECD 405
Skin sensitization	OECD 406
Repeated dose 28-day oral toxicity study in rodents	OECD 407
Repeated dose 90-day oral toxicity study in rodents	OECD 408
Combined chronic toxicity/carcinogenicity studies	OECD 453
Combined repeated dose 90-day and 12-month oral toxicity study in non-rodents	OECD 409
Repeated dose inhalation toxicity: 28-day or 14-day study	OECD 412
Subchronic dermal toxicity: 90-day study	OECD 411
Bacterial reverse mutation test	OECD 471
<i>In vitro</i> mammalian chromosomal aberration test	OECD 473
<i>In vitro</i> mammalian cell gene mutation test	OECD 476
Mammalian erythrocyte micronucleus test	OECD 474
Unscheduled DNA synthesis (UDS) test with mammalian liver cells <i>in vivo</i>	OECD 486
Carcinogenicity studies	OECD 451
Two-generation reproduction toxicity study	OECD 416
Prenatal developmental toxicity study	OECD 414
Neurotoxicity study in rodents (acute)	OECD 424
Neurotoxicity study in rodents (subchronic)	EPA/OPPTS 870.6200

OECD, Organization for Economic Co-operation and Development – Chemicals Testing – Guidelines Section 4 <http://new.sourceoecd.org/v1=7455433/cl=12/nw=1/rpsv/cw/vhosts/oecdjournals/1607310x/v1n4/contp1-1.htm>.

EPA/OPPTS, US Environmental Protection Agency/Office of Prevention, Pesticides and Toxic Substances—Series 870 Health Effects Test Guidelines (http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/index.html).

administered by gavage, while dogs received the test substance via capsules. Studies with single administration were performed at the limit dose given in the respective guideline. In studies with repeated administration the dose levels were selected based on range finding studies. According to the guideline requirements, the high-dose levels were selected with the aim to induce toxic effects but not death or severe suffering. Thereafter descending sequences of dose level were selected to demonstrate any dosage-related response and NOAEL at the lowest dose level. Generally, at least three test groups and one control group were used. The parameters investigated in the studies were defined by the guidelines and depend on the study type, but always included

general observation of the animals, clinical examination, clinico-chemical and hematological examinations, as well as necropsy of all animals at the end of the treatment period and histopathology of defined groups.

2.1. Toxicokinetics and metabolism

After oral administration of ^{14}C -metaflumizone to male and female rats, labeled either in the benzonitrile-ring (B-label) or in the trifluoromethoxyphenyl-ring (T-label) of the molecule, absorption of metaflumizone was shown to be relatively low with a reverse, dose-dependent bioavailable amount of ^{14}C -metaflumizone of up to 17% of the administered dose at a dose rate of 6 mg/kg bw. The major route of excretion was via feces (up to 90% of dose) and only minor percentages of the administered dose were excreted via bile and urine.

In pharmacokinetics experiments, following a single oral dose of ^{14}C -metaflumizone, the maximum plasma concentrations were reached after 10–48 h (T_{\max}), depending on the dose and the radio-label tested. The increase of the dose by a factor of 33 resulted in an increase of the area under the curve ($\text{AUC}_{0\rightarrow\infty}$) of about 10, correlating with the lower bioavailability of metaflumizone at the higher dose. The absorbed ^{14}C -metaflumizone was distributed by systemic circulation to all organs and tissues. Total radioactive residues in tissues reached maximum levels at or near the T_{\max} in both male and female rats irrespective of dose rates, with the highest concentration detected in fat/liver followed by kidney and blood/plasma/muscle.

The elimination half-lives depended on the radio-label position, ranging from 27–48 to 139–402 h for the B-labeled and the T-labeled ^{14}C -metaflumizone, respectively.

The absorbed ^{14}C -metaflumizone was metabolized by the rat via hydrolysis and hydroxylation, and typical phase two reactions were conjugation reactions with sulfate or glucuronic acid, while substitutions with glutathione were less common.

^{14}C -metaflumizone was also administered to rats in a single dermal application in order to give information about dermal penetration of metaflumizone. For this purpose, ^{14}C -metaflumizone (B-label) was applied dermally in an aqueous preparation (suspension concentrate formulation) at two different dose levels for 6 h and different time points of post-observation. The high dose tested represented the concentration of the formulation concentrate for crop protection applications, while the low dose corresponded to the dilution of metaflumizone that might be used in the field (spray dilution). The values obtained for dermal penetration of

metaflumizone in this type of formulation were very low. The dermal absorption for the high dose was 0.08% of the applied dose after a 6-h exposure. After post-exposure periods of 24 and 120 h, the values were in the same order of magnitude (0.11–0.13%). Dermal absorption was also very low for the low dose with 0.64% absorbed at 6 h, and post-exposure values of 1.02 and 1.70% after 24 and 120 h, respectively.

2.2. Acute toxicity

Metaflumizone is virtually non-toxic to Sprague–Dawley rats after oral and dermal application, with LD₅₀ values > 5000 mg/kg. At this limit dose tested, metaflumizone caused neither mortality nor systemic toxicity via either the oral or dermal routes. In addition, metaflumizone was of low acute toxicity via the oral route to albino mice with an LD₅₀ value > 5000 mg/kg. The inhalation toxicity of metaflumizone as a dust aerosol was also low when tested in a nose-only, inhalation toxicity limit test in Wistar rats (4 h LC₅₀ > 5.2 mg/l). Metaflumizone was not irritating to the skin of rabbits and was non-to-slightly irritating to the eye of rabbits. Metaflumizone has no potential to induce skin sensitization as confirmed by a negative result in the Guinea pig maximization test.

2.3. Subchronic toxicity studies

Metaflumizone was tested for its short-term oral toxicity in rats in a 28-day/13-week oral gavage range finding study and in a 90-day oral gavage study (90-day timepoint in the chronic toxicity/carcinogenicity study). In dogs, short-term toxicity was investigated in a combined 90-day/12-month study.

In the Sprague–Dawley rat, treatment by oral gavage at dose levels of 0, 30, 60 and 300 mg/kg bw with metaflumizone for a subchronic duration resulted in reduced food consumption and/or decreased mean body weight and/or decreased body weight gains in males and females at the highest dose tested and a mild lesion in the livers of males at the same dose. Under the conditions of the study, the no observed adverse effect level (NOAEL) for oral administration of metaflumizone for 90 days was 60 mg/(kg day).

In Beagle dogs administered metaflumizone orally by gelatin capsule for periods of 3 and 12 months, effects were observed on food consumption at dose levels greater than and including 30 mg/(kg bw day). Additionally, reduced body weight gain and body weight loss were noted, leading to poor general state of health of several animals. Some changes in hematological parameters

were also assessed to be substance-related. Microscopic findings in some organs of individual, prematurely sacrificed animals were clearly related to the body weight loss, and therefore assessed to be secondary in nature. Females were generally affected more often and to a higher extent in this study. The NOAEL for male and female Beagles in this combined 3-month/12-month toxicity study was 12 mg/(kg day).

Additionally, metaflumizone was tested for its short-term toxicity in rats via other routes of exposure in a 28-day inhalation toxicity study (nose-only), a supplemental inhalation toxicity study (whole body) and a 90-day dermal toxicity study.

In the 28-day inhalation study, rats were exposed to metaflumizone dust aerosol atmospheres for 6 h per working day in a nose-only dynamic inhalation chamber. This exposure regime led to pronounced effects at concentration levels greater than and including 100 mg/(m³ day). In addition to inanition associated with exposure to the test article, “restraint” stress associated with nose-only exposure is regarded as the triggering stress factor for the animals, resulting in severe body weight loss and causing a number of indirect (secondary) effects. Furthermore, treatment-related local effects indicating some irritation by the presence of dust particles were observed in the nasal cavity and lungs at these concentrations. There were no treatment-related effects observed at the low concentration of 30 mg/(m³ day).

In order to differentiate primary toxicity from secondary effects associated with the nose-only exposure method, an additional inhalation study was performed using whole-body exposure. Importantly, the actual exposure of the animals was higher than the target atmosphere concentration of 30 mg/m³, due to additional dermal and oral exposure that occurs by whole body exposure.

Four weeks of inhalation of a dust aerosol at a target atmosphere concentration of 30 mg/m³ of metaflumizone led to moderately decreased food consumption and a corresponding marked reduction of body weight gain in male and female animals. However, there was a lack of systemic toxicity noted except for some indirect effects. Taking into account the results of this supplemental whole body exposure study, the no observed adverse effect concentration (NOAEC) for inhalation toxicity is considered to be 30 mg/(m³ day).

Lastly, in a 90-day dermal toxicity study conducted with metaflumizone technical in Wistar rats, the results support a NOAEL of 100 mg/(kg day), based on decreased food consumption (females) and decreased

body weight change in males and females at the next higher dose tested. There were no signs of local irritation noted up to the limit dose.

2.4. Chronic toxicity/carcinogenicity studies

The chronic toxicity/oncogenicity studies with metaflumizone included a combined oral rat chronic/oncogenicity gavage study (12 and 24 months) in Sprague–Dawley rats, and an 18-month gavage study in CD-1 mice.

The administration of metaflumizone to Sprague–Dawley rats for a 2-year chronic duration at dose levels of 0, 30, 60 and 300 mg/(kg bw day) by gavage resulted in dose-related microscopic liver lesions in males and females. Treatment with metaflumizone for 24 months to Sprague–Dawley rats resulted in no test substance-related neoplastic findings at any dose. Therefore, under the conditions of this study, the NOAEL for systemic toxicity following oral administration of metaflumizone for 24 months to Sprague–Dawley rats was 30 mg/(kg day) for male and female rats.

The administration of metaflumizone to CD-1 mice for an 18-month chronic duration at dose levels of 0, 100, 250, and 1000 mg/(kg bw day) by gavage resulted in dose-related higher incidences of increased brown pigment in the spleen of male and female animals at the highest dose level only. No test substance-related neoplastic findings were observed up to the highest dose tested. Thus, under the conditions of this study, the NOAEL for systemic toxicity in mice was 250 mg/(kg day), while the NOAEL for carcinogenicity was >1000 mg/(kg day) (highest dose tested).

2.5. Mutagenicity/genotoxicity studies

Metaflumizone was tested in a battery of *in vitro* and *in vivo* assays measuring several different endpoints of potential genotoxicity such as gene mutation, chromosomal aberration and DNA damage/repair. Specifically, for the battery of three *in vitro* mutagenicity assays with metaflumizone, no positive responses were observed for increased revertant frequencies with and without metabolic activation in both the bacterial reverse mutation assay and in an *in vitro* genotoxicity test in mammalian cells. Although there was a positive result for a statistically increased number of structurally aberrant metaphases in the chromosomes, which indicates clastogenic potential under *in vitro* conditions, this result was only observed without metabolic activation in a cytogenicity study with mammalian cells. Importantly, the potential biological significance

of this apparent chromosome damage observed *in vitro* only, without metabolic activation, was evaluated in a higher tier *in vivo* study using the mouse micronucleus assay. This study in mice was conducted at a high-dose level that demonstrated clinical symptoms of toxicity. In the kinetic study it could be demonstrated that the test article reached the target organ (bone marrow). No significant or dose-related increases in chromosomal damage were observed in this *in vivo* test, indicating that metaflumizone does not cause chromosomal aberrations in intact animals. In addition, a second *in vivo* study in Wistar rats, investigating unscheduled DNA synthesis, did not reveal any effects.

2.6. Reproductive and developmental toxicity studies

Metaflumizone was tested for its potential reproduction toxicity in a two-generation reproduction toxicity study in rats, in a prenatal developmental toxicity study in rats, and in a prenatal developmental toxicity study in rabbits.

In the two-generation reproduction toxicity study in Wistar rats, where the subject animals were administered the product by oral gavage, the highest dose tested (75 mg/(kg bw day)) induced both excessive maternal as well as developmental toxicity, which together resulted in high pup mortality. Consequently, a meaningful assessment of the potential reproductive toxicity of the test compound at this excessively toxic dose level was not possible. Thereafter, for the next two successive parental generations of rats, the highest dose tested was reduced to 50 mg/(kg bw day).

Subsequently, the NOAEL for parental toxicity was determined to be 20 mg/(kg day), based on increased incidences of poor general health and reduced body weights for females at the highest dose tested for two consecutive generations. The NOAEL for pup toxicity was considered to be 20 mg/(kg day), based on a slightly increased incidence of pup mortality at the high-dose level tested. The NOAEL for fertility was the high-dose level (50 mg/(kg day)) for two consecutive generations, the NOAEL for reproductive performance was considered to be 20 mg/(kg day).

In the oral (gavage) developmental toxicity study in rats, administration of metaflumizone to pregnant Wistar rats from implantation to 1 day prior to the expected day of parturition (Days 6–19 post-coitum) at dose levels of 0, 15, 40 and 120 mg/(kg bw day), evoked signs of maternal toxicity, as reductions in food consumption, and impairments in absolute and corrected body weight gains, at the highest dose tested

(120 mg/(kg day)). No signs of substance-induced maternal toxicity occurred at the other dose levels. There were no substance-related influences on the gestational parameters up to and including the highest dose level. The administration of metaflumizone to the dams caused no signs of developmental toxicity and in particular no indications of teratogenicity at any of the dose levels tested. Placental and fetal body weights were unaffected. The external, soft tissue and/or skeletal (including cartilage) examinations of the fetuses revealed no biologically relevant differences between the control and the substance-treated groups. The NOAEL, derived from this study for prenatal developmental toxicity in the rat, therefore, was 120 mg/(kg day) (highest dose tested). Metaflumizone is considered to be neither a developmental toxicant nor a teratogenic agent in the rat.

In the prenatal developmental toxicity study in rabbits, the oral administration of metaflumizone by gavage at dose levels of 0, 30, 100 and 300 mg/(kg bw day) to pregnant Himalayan rabbits from implantation to 1 day prior to the expected day of parturition (Days 6–28 post-insemination) elicited overt signs of maternal toxicity based on several clinical symptoms of toxicity (poor general state including ataxia) occurring in four of 25 does at the high-dose tested (300 mg/(kg day)), for which two of these four does had abortions prior to being sacrificed early, with a third doe being sacrificed moribund. As such, the NOAEL for maternal toxicity was 100 mg/(kg day). Similarly, the NOAEL for developmental (fetal) toxicity was 100 mg/(kg day), based on slightly (non-statistically) decreased mean fetal body weight, primarily due to increased number of runts in two litters at 300 mg/kg bw/day (highest dose tested). In addition, the incidence of the skeletal variation of incomplete ossification of sternaebrae (considered to be transient and fully reversible post-natally) was statistically increased at the highest dose tested, compared to control. Importantly, since the NOAEL's for both maternal and fetal toxicity are the same (100 mg/(kg day)), metaflumizone was not selectively toxic to the developing fetal rabbit. Lastly, because there were no indications of any teratogenic effects in the rabbit fetuses at the highest dose tested, the compound is not considered to be teratogenic in the rabbit.

2.7. Neurotoxicity studies

The potential neurotoxicity of metaflumizone was investigated in an acute oral and a 90-day oral neurotoxicity study in Wistar rats. Administration of

metaflumizone in an acute neurotoxicity study at dose levels of 0, 125, 500 and 2000 mg/kg bw in male and female rats did not lead to signs of general toxicity or neurotoxicity up to the highest dose tested. Therefore, the NOAEL in this study was 2000 mg/kg for male and female rats.

Administration of metaflumizone to rats by oral gavage at dose levels of 0, 12; 36; 150 (both sexes) and 300 (males only) mg/(kg bw day) in a subchronic (3 months) neurotoxicity study resulted in indications of general systemic toxicity at the mid-dose and high-dose groups. However, only one male animal of the mid-dose group demonstrated clinical findings of a poor general state and had findings at necropsy. The findings related to this animal were indicative of an infection, and therefore, could be considered incidental and not related to test substance administration. This is further substantiated by the fact that another male animal at the higher dose level had the same clinical findings at the same time period without leading to mortality. No clinical or neuropathological signs of neurotoxicity were detected within the entire study at any dose level. Thus, under the conditions of this study, the NOAEL's regarding neurotoxicity correspond to 300 mg/(kg day) in males and 150 mg/(kg day) in females (the highest doses tested, respectively).

3. Discussion

Results from toxicokinetic studies using oral administration, indicate that up to 17% of the metaflumizone applied may be absorbed in a reverse, dose-dependent manner. For the most part, orally administered metaflumizone is excreted via the feces and to a large extent is not systemically bioavailable. The portion that is absorbed, becomes rapidly distributed throughout the body, and is extensively metabolized.

The skin represents an effective barrier for metaflumizone. Only very minor fractions of an applied amount penetrated through the dermis, when metaflumizone was applied as an aqueous preparation.

Metaflumizone has low acute toxicity in rats via the oral, dermal and inhalation routes of exposure. It is not an irritant to rabbit skin and eyes and not a skin sensitizer in Guinea pigs. This characteristic of being virtually non-toxic regarding acute toxicity is an outstanding property of metaflumizone, which is especially exceptional for an insecticide.

The short-term and long-term toxicity profile of metaflumizone is mainly characterized by decreased body weight, body weight gain and food consumption

via all routes tested (oral, inhalation and dermal). Findings in clinico-chemical and histopathological examinations correlated to the degree of impairment of body weight change. Using an overall weight-of-evidence approach, metaflumizone is not considered to be mutagenic or genotoxic. The single positive response observed in the *in vitro* chromosome aberration assay, in absence of metabolic activation, was not confirmed in a corresponding higher tier *in vivo* assay, proving that metaflumizone does not lead to chromosomal aberrations in animals. Importantly, no neoplastic potential of the test substance was either observed in rats or in mice up to the highest dose tested.

With regard to potential reproductive toxicity and teratogenicity, results of the two-generation reproductive toxicity study as well as the rat and rabbit developmental toxicity studies, indicate that the active ingredient is neither a reproductive toxicant nor a teratogen, and is not selectively toxic to the fetal rat or rabbit.

Specific studies investigating neurotoxicity revealed the lack of any neurotoxic potential of metaflumizone.

4. Conclusion

The test battery performed on metaflumizone provides a comprehensive view of the toxicological profile of the substance. Overall, metaflumizone showed an exceptionally low potential for acute toxicity and a relatively low potential to cause adverse effects in mammals after short- and long-term exposure. For all relevant endpoints, clear NOAEL's and thus, reference values could be established for the performance of acute, short-term and chronic risk assessments to humans.

Acknowledgement

The authors would like to thank Theodora Wang for her assistance.