

## TOXICOLOGICAL SUMMARY FOR SULFLURAMID TOXICITY TO SUPERIOR ANIMALS

- I) Acute oral toxicity evaluation on rats.  
LD 50 Oral in rats = 6,600 mg/kg  
Reference: Certificado Oficial de Análise (Official Analysis Certificate) n° 6828. Série AG – CEPPA – Centro de Pesquisa e Processamento de Alimentos – Universidade Federal do Paraná (Federal University of Paraná).
- II) 90-days Sub Chronic Dietary Toxicity Study in Rats, Sulfluramid (MRD-89-472): Exxon Biomedical Sciences, Inc. Study No. 247254.  
Sulfluramid was administered via dietary admixture to groups of Sprague-Dawley albino rats for thirteen weeks. The dietary levels were 0 (Control), 10 (low dose), 50 (mid dose) and 150 (high dose) ppm.  
Ten animals (6 male, 4 female) in the high (150 ppm) dose group died prior to study termination. Clinical in-life observations noted during the test period were minimal. Some high dose animals showed agitation, convulsions, hyperactivity and an increased incidence of emaciation.  
Statistically significant decreases in body weight were noted in high dose males and females throughout the study. Decreased body weight was noted in the mid-dose females from Week 7 through termination. Significantly decreased food consumption was seen occasionally in high dose males and mid dose females and at all food consumption intervals for high dose females.  
Hematology measurements revealed statistically significantly decreased red blood cell counts, hematocrit and hemoglobin values in high dose males and females. Consistent serum chemistry changes included increased blood urea nitrogen and alkaline phosphatase values for the mid dose males and high males and females. Decreased cholesterol was noted in mid dose females and high dose males and females. Increased albumen and total protein were also noted in high dose males while high dose females also showed increased total bilirubin, inorganic phosphorus, sodium and alanine aminotransferase.  
Terminal necropsy findings in the mid and high dose animals included emaciation, stomach discoloration, enlargement and discoloration of the liver and lung discoloration.  
Increased absolute and relative liver weights were seen for mid dose females and high dose males and females. The relative liver weight for the mid dose males was also increased over controls. Other organ weight differences appeared to be related to the body weight losses seen in the mid dose females and the high dose males and females.  
There were no biologically important or statistically significant differences for sperm motility or spermatid concentration values from treated males as compared with control group values. No treatment related effects were observed at the terminal ophthalmoscopic examination.  
No treatment-related microscopic changes were seen in any of the low dose males and females. Treatment-related microscopic changes in the mid and high dose male and female rats included centrilobular hepatocellular hypertrophy and midzonal (males) or periportal (females) hepatocytic

vacuolation as well as focal necrosis or hemorrhage of the gastric glandular mucosa. An increased incidence of multifocal renal medullary mineralization was noted in mid and high dose female rats.

Based on the results of this study, a dietary level of 10 ppm Sulfluramid is considered as the NOEL when administered to rats for 90 days.

III) Sulfluramid Acute Inhalation Toxicity Study – LC50 Rats (4 Hour Exposure), Hazleton UK, Study No. 6443-788/1.

Five male and five female Sprague Dawley rats were exposed for 4 hours to an Sulfluramid aerosol concentration of 4.379 mg/l. The sulfluramid was dissolved in acetone so that chamber delivery was possible. This Sulfluramid concentration represented the maximum practical in view of the potential flammability and toxicity of the vehicle. No deaths occurred during the exposure or the 14 day post exposure observation period. It was concluded that the LC50 was greater than 4.379 mg/l.

IV) Acute dermal toxicity evaluation on rats.

LD 50 Dermal in rats > 2,000 mg/kg

Reference: Certificado Oficial de Análise (Official Analysis Certificate) n° 6812. Série AG – CEPPA – Centro de Pesquisa e Processamento de Alimentos – Universidade Federal do Paraná (Federal University of Paraná).

V) Acute Exposure Dermal Toxicity, Pharmakon Research International, Study N° PH 422-GC-001-90.

Ten rabbits (five males and five females) were dermally administered Sulfluramid at dose of 2.000 mg/kg. No animals died during this study. Based on the results of this study, the dermal LD50 was considered to be greater than a limit dose of 2000 mg/kg.

VI) Acute Dermal Irritation / Corrosion in rabbits.

Not irritant.

Reference: Certificado Oficial de Análise (Official Analysis Certificate) n° 6941. Série AG – CEPPA – Centro de Pesquisa e Processamento de Alimentos – Universidade Federal do Paraná (Federal University of Paraná).

VII) Acute Eye Irritation / Corrosion in rabbits.

Caused irritation, without corneal opacity and was reversible in up to 72 hours.

Reference: Certificado Oficial de Análise (Official Analysis Certificate) n° 6817. Série AG – CEPPA – Centro de Pesquisa e Processamento de Alimentos – Universidade Federal do Paraná (Federal University of Paraná).

VIII) Dermal Sensitization, in guinea pigs (Draize's method).

Not sensitizing.

Reference: Certificado Oficial de Analise (Official Analysis Certificate) n° 6923. Série AG – CEPPA – Centro de Pesquisa e Processamento de Alimentos – Universidade Federal do Paraná (Federal University of Paraná).

IX) Genotoxic Potential.

a. Prokaryotes

Mutagenicity Test on GX-071 (Sulfluramid) in the Ames Salmonella / Reverse Mutation Assay, Hazleton Laboratories America Study N° 10549-0-401.

GX-071 was evaluated for mutagenic activity in an Ames Salmonella / Microsome assay using Salmonella typhimurium strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538. The assays were conducted using triplicate plates per dose level and in the presence and absence of a metabolic activation system. GX-071 did not exhibit genetic activity in these assays and was not mutagenic under the test conditions of this assay.

b. Eukaryotes

Mutagenicity Test on Sulfluramid (GX-071) in the Rat Primary Hepatocyte Unscheduled DNA Synthesis Assay, Hazleton Laboratories America Study N° 10549-1-447.

Sulfluramid was evaluated for potential effects in a Unscheduled DNA Synthesis Assay. An independent repeat assay was also conducted to tighten the dose range for this test. Sulfluramid was considered inactive in both the initial and the repeat assays.

X) Embryonic Potential

Developmental Toxicity Study in Rats with Sulfluramid, Pharmakon Research International Study N° PH 328-GC-001-90

Sulfluramid was administered orally, by gavage, to pregnant female rats on days 6 through 15 of gestation at doses of 1 (low), 4 (mid) and 16 (high) mg/kg/day. A concurrent vehicle control group was administered corn oil at a dose volume of 5 ml/kg/day. Cesarean section was performed on each dam on Day 20 post copulation.

There were no treatment related clinical signs of toxicity observed in the vehicle control, low or mid dose groups. Clinical signs noted in the high dose dams included poor grooming, decreased activity, piloerection, alopecia, flaccid body tone, abnormal gait and abnormal stance. None of the dams died during the study.

Lower than control mean body weight gain was observed for the mid dose dams while high dose dams showed weight losses during the dosing period. The high dose group exhibited a significant increase in body weight during the Days 15-20 gestation interval. Decreased food consumption was also noted for the 16 mg/kg/day dose group during most food consumption intervals.

No differences were noted in the total number of implantation sites, total number of viable fetuses, early resorptions, late resorptions, fetal sex

distribution, corpora lutea, the number of and percentage of pre-implantation or post-implantation losses. Lower mean fetal body weight was observed in the 16 mg/kg/day dose group.

No fetal deaths were recorded at cesarean section. In the high dose, 12 of 339 fetuses from 4 of 24 litters exhibited skeletal or visceral malformations. These findings were statistically significantly higher than the control group and were considered to be the result of the severe maternal toxicity noted in the dams at this dose.

Based upon these findings, the no-observed effect level (NOEL) for maternal toxicity is considered to be 1 mg/kg/day and no-observable effect level for developmental toxicity is 4 mg/kg/day.

Developmental Toxicity Study in Rabbits with Sulfluramid,  
Pharmakon Research International Study N° PH 329-GC-001-90

Sulfluramid was administered orally, by gavage, to artificially inseminated New Zealand white female rabbits on days 6 through 18 of gestation at doses of 0.1 (low), 0.5 (mid) and 1.5 (high) mg/kg/day. A concurrent vehicle control group was administered 2.0% carboxymethylcellulose at a dose volume of 3 ml/kg/day. Cesarean section was performed on each dam on Day 29 post insemination.

No treatment related clinical signs were observed. No statistically significant differences were observed in mean maternal body weights or body weight changes. However, the body weight gain for the mid and high dose groups were approximately 20-30% of control body weight (i.e., 70 to 80% suppression) during the Days 6-18 dosing interval. There were no statistically significant differences observed in the amount of feed consumed among the four groups.

There was no statistically significant differences in the total number of implantation sites, total number of viable fetuses, non-viable fetuses, early resorptions, late resorptions, fetal sex distribution, fetal crown-rump length, fetal body weight, corpora lutea, the number and percentage of pre-implantation or post-implantation losses.

There were a total of 13 fetal deaths at cesarean section: nine (9,2%) in the control group, one (1,3%) in the 0,5 mg/kg/day dose group and three (2,9%) in the 1.5 mg/kg/day dose group. Seven of the nine dead fetuses in the control group were noted in one dam. The deaths judged coincidental and not the result of treatment with the vehicle or Sulfluramid.

There were no statistically significant differences between the treated and control groups in the number of fetuses or litters with skeletal, soft tissue or external malformations.

Maternal toxicity was observed in the 0,5 and 1,5 mg/kg/day dose groups as evidenced by suppressed body weight gain during the treatment interval. Fetal viability, body weight and morphology were not adversely affected at any dose level.

Based upon these findings, the NOEL for maternal toxicity is considered to be 0,1 mg/kg/day and the NOEL for developmental toxicity is 1,5 mg/kg/day.

- XI) Evaluation of the promoting potential of Sulfluramid HB Thechnical in the medium-term multi-organ carcinogenesis bioassay. Núcleo de Avaliação Toxicogenética e Cancerígena – TOXICAN. Departamento de Patologia – Faculdade de Medicina UNESP – Botucatu.

The carcinogenic potential of a 30/70 Sulfluramid mixture was evaluated in Wistar rats of both sexes through a medium duration test in multiple organs based on the concept of initiation/promotion carcinogenesis. After initiation of the test animals with subcarcinogenic doses of five initiating agents, the animals were exposed to doses of 0, 10, 30 and 90 ppm Sulfluramid. Additional groups of animals not receiving the initiating agents were also exposed to these same Sulfluramid concentrations. Groups of initiated animals were also treated with positive control substances (phenobarbital and 2"AAF). Dietary administration of 90 ppm Sulfluramid (approximately 9,5 mg/kg/day) in this assay for 25 weeks led to the development of benign neoplasia in the liver of the initiated male rats only. The higher incidence of liver neoplasia in the initiated males was associated with increased liver weights, hepatocytic hypertrophy, hyperplasia of the biliary ducts and focal steatosis. Similarly, liver toxicity characterized by hepatocytic hypertrophy, focal steatosis and eosinophilic and basophilic hepatic foci were seen in non-initiated males receiving 90 ppm Sulfluramid; no evidence of neoplasia was seen in these animals. Although significant body weight reduction and increased absolute and relative liver weights were evident for 90 ppm initiated and non-initiated females, Sulfluramid did not induce evidence of hepatotoxicity in these animals. No evidence of neoplasia occurred in the females at this dose nor in animals of both sexes receiving 10 or 30 ppm. These results clearly demonstrated that the hepatic neoplasia seen in initiated male rats at 90 ppm was related to the sex-related hepatotoxicity at a Sulfluramid dose which clearly exceeded an acceptable MTD. Numerous studies have shown that doses greater than the MTD induce alterations in xenobiotic metabolism and homeostatic mechanisms which compromise the relevance of the result for cancer risk assessment. The 30 ppm dose level used in this study more closely meets current acceptable standard for a MTD. No evidence of neoplasia was seen at this dose level. Therefore, Sulfluramid was not considered to be carcinogenic under the conditions of this study.

#### TOXICITY FOR NON-TARGET ORGANISM

- XII) Acute toxicity to *Spirillum volutans*.  
The minimal effective rated concentration that causes the loss of typical reverse movement and/or inactivation of the bacterial cell in 90% of the bacteria after 30 minutes is 0.1585 g/100 ml, with MEC 90 (30') of 90% and the TU (Toxic Unity) = 1.1.  
Reference: Certificado Oficial de Análise (Official Analysis Certificate) n° 1328. Série AG – CEPPA – Centro de Pesquisa e Processamento de Alimentos – Universidade Federal do Paraná (Federal University of Paraná).
- XIII) Acute toxicity appraisal of the product Sulfluramid HB Técnico in *Selenastrum capricornutum*.  
CEI 50, 96 hours: 68.12 mg/l.  
Confidence interval (95%): 60.90 to 76.20 mg/l.  
Minimal concentration that causes total inhibition: greater than 100 mg/l.  
Maximal concentration that not causes growing inhibition: 10 mg/l.  
Results obtained allow the product to be classified as Class III – Slightly toxic.  
Reference: Certificado Oficial de Análise (Official Analysis Certificate) n° 2077. Série AG – CEPPA – Centro de Pesquisa e Processamento de Alimentos – Universidade Federal do Paraná (Federal University of Paraná).
- XIV) Toxicity evaluation on soil organisms. Worm – *Eisenia foetida*.  
Observed mortality in white test: no mortality observed in organisms after the 14th test day.  
Mean initial lethal concentration (LC (I) 50) of chloracetamide: 49.24 mg/kg. Confidence Limits 41.33 - 58.68 mg/kg.  
Lowest concentration of test substance at which observed lethality was 100% during the 14 days of test: 400 mg/kg.  
Highest concentration of test substance at which no lethality was observed: 100 mg/kg.  
Mean initial lethal concentration (LC (I) 50) of test substance: 246.2288. Confidence Limits: 206.63 – 293.42  
Reference: Certificado Oficial de Análise (Official Analysis Certificate) n° 1245. Série AG – CEPPA – Centro de Pesquisa e Processamento de Alimentos – Universidade Federal do Paraná (Federal University of Paraná).
- XV) Toxicity in bees (*Apis mellifera*).  
This study was carried out to determine the acute contact toxicity to honeybees (*Apis mellifera*) of the product TECHNICAL SULFLURAMIDE HB (AKI 001-95). Workerbees in five groups of ten were topically dosed with the following doses: 0,01 µg/bee; 0,10 µg/bee; 1,00 µg/bee; 10,0 µg/bee and 100 µg/bee. Tween 80 (0,1 µg/bee) was employed for solubilization of the test substance. All doses were tested in triplicate. After 48 hours exposition, the nominal dose that causes 50% mortality of animals was established. The median lethal contact dose (LD50) and 95% fiducial limits were estimated using trimmed Spearman-Kärber method (Hamilton et alii, 1977). The toxicity of the

product AKI 001-95 to honeybees (*Apis mellifera*), under the test conditions and based on nominal dose was 38,4 µg/bee with 95% fiducial limits between 30,7 µg/bee and 48,0 µg/bee.

Reference: Report # RL 52.857. TECAM – Tecnologia Ambiental.

XVI) Acute Toxicity of GX-071 to *Daphnia magna*, ABC Laboratories Study N° 37184.

The 48 hour EC50 for GX-071 (Sulfluramid) in *Daphnia magna* was determined to be greater 10 mg/l (analytically measured).

This was the highest concentration (including use of a cosolvent) that could be achieved due to low water solubility. The NOEL was determined to be 10 mg/l.

XVII) Determination of chronic toxicity in *Ceriodaphnia dubia*

CENO = 0,15 mg/l

CEO = 0,60 mg/l

CV = 0,30 mg/l

Reference: Work performed by Bioensaios Análise e Consultoria Ambiental Ltda.

XVIII) Acute Toxicity Test in Fish

Acute Toxicity of GX-071 to Bluegill (*Lepomis macrochirus*), ABC Laboratories Study N° 38276.

The 96-hour LC50 for GX-071 (Sulfluramid) in Bluegill was determined to be greater than 6.6 mg/l (analytically measured). This was the highest concentration (including use of cosolvent) that could be achieved due to low water solubility. The NOEL was determined to be 1.6 mg/l.

Acute toxicity of GX-071 to Rainbow Trout (*Salmo gairdneri*), ABC Laboratories Study n° 37183

The 96-hour LC50 for GX-071 (Sulfluramid) in Rainbow trout was determined to be greater 10 mg/l (analytically measured). This was the highest concentration (Including use of a cosolvent) that could be achieved due to low water solubility. The NOEL was determined to be 10 mg/l.

XIX) Chronic Toxicity Test in Fish

CENO = 3,2 mg/l

CEO = 5,8 mg/l

CV = 4,31 mg/l

Reference: Report # 1016/108 - Biomesos Assessoria Farmacológica.

XX) Bioconcentration in fish (*Brachydanio rerio*).

This study was carried out to determine the accumulation potential of the product TECHNICAL SULFLURAMIDE HB (AKI 001-95) in the tissues of *Brachydanio rerio*. One hundred animals were exposed to the average concentration of 8 µg/L of test substance for 14 days in semi-static system (uptake phase). Samples of water and fishes were periodically collected during this period (total of six samples). Then, animals were kept in clean water for seven days (clearance phase) and four samples were collected. According to the bioconcentration model adopted, the rate constant of uptake (k1) was calculated in 53,7 L/g/h, the rate of clearance (k2) in 0,05/h and the Bioconcentration Factor (BCF) in 1131.

Reference: Report # RL 833.434. TECAM – Tecnologia Ambiental.

XXI) Avian Oral LD50 Study in Bobwhite Quail, University of Georgia Study N° UGA 013

Based on the results of this study, the acute oral LD50 of GX-071 (Sulfluramid) in bobwhite quail was calculated to be 473,76 mg/kg.

XXII) GX-071 - A Dietary LC50 Study with the Mallard, Wildlife International Study N° 207-102A.

The dietary LC50 of GX-071 (Sulfluramid) was determined to be 165 ppm with confidence limits of 100 to 316 ppm.

XXIII) Avian Subacute Dietary toxicity – Bobwhite Quail

Sulfluramid Technical: 8-Day Acute Dietary LC50 Study in Bobwhite Quail, Bio-Life Associates Study N° 90 QC 151.

The dietary LC50 of Sulfluramid was determined to be 300 ppm with confidence limits of 256 to 351 ppm.

TOXICITY OF ANT BAIT (SULFLURAMID 0.3%).

I) Acute oral toxicity evaluation in rats.

LD 50 Oral in rats > 2,000 mg/kg.

Reference: Certificado Oficial de Analise (Official Analysis Certificate) n° 6697. Série AG – CEPPA – Centro de Pesquisa e Processamento de Alimentos – Universidade Federal do Paraná (Federal University of Paraná).

II) Acute dermal toxicity evaluation in rats.

LD 50 Dermal in rats > 2,000 mg/kg

Reference: Certificado Oficial de Analise (Official Analysis Certificate) n° 6687. Série AG – CEPPA – Centro de Pesquisa e Processamento de Alimentos – Universidade Federal do Paraná (Federal University of Paraná).

III) Acute dermal irritation / corrosion in rabbits.



Not irritant.

Reference: Certificado Oficial de Análise (Official Analysis Certificate) nº 6685. Série AG – CEPPA – Centro de Pesquisa e Processamento de Alimentos – Universidade Federal do Paraná (Federal University of Paraná).

IV) Acute eye irritation / corrosion in rabbits.

Minimal irritation reversible within 24 hours.

Reference: Certificado Oficial de Análise (Official Analysis Certificate) nº 6688. Série AG – CEPPA – Centro de Pesquisa e Processamento de Alimentos – Universidade Federal do Paraná (Federal University of Paraná).

V) Reversed gene mutation (Ames test).

The reverse mutation assay (Ames Test) was carried out with the product AKI 004-96 in order to study the possible mutagenic effect of that substance on the strains TA98; TA100; TA1535; TA1538 of *Salmonella typhimurium* in systems with and without metabolic activation (microsomal fraction of rat liver induced with Aroclor 1254). The definitive test was performed on strains TA98; TA100; TA1535; TA1538 with the following concentrations: 648; 1080; 1800; 3000; 5000 µg/plate of AKI 004-96. Mutation frequencies after 72 hours of incubation of the three *Salmonella* strains exposed to AKI 004-96 were lower than 2. Statistical analysis using the software "Salanal" presented no significant results (estimate slope < 0,01). Under the conditions of this study, AKI 004-96 has presented no mutagenic effect on *Salmonella typhimurium* strains TA98; TA100; TA1535; TA1538 both with and without metabolic activation.

Reference: Report # RL 722.079. TECAM – Tecnologia Ambiental.

VI) Micronucleous in mouse bone marrow

RESULT: No significant increase in the micronucleus number was observed in 20.000 polychromatic erythrocytes analysed of the animals treated with 2000 mg/kg of AKI 004-96 when compared to negative control.

CONCLUSION: Under the conditions of this study, AKI 004-96 did not induce an increase of micronucleus number in mouse bone marrow erythrocytes.

Reference: Report # RL 103.021. TECAM – Tecnologia Ambiental.

VII) Toxicity in soil microorganisms involved in nitrogen cycle.

The GX 439-0.3% SULFLURAMID 70/30 product was tested in laboratory in order to appraise its toxicity on soil microorganisms involved in the nitrogen cycle. During periods of 7, 14, and 21 days, in two soil classes (LVe and PVa), the agronomically recommended dosage (D.A.R. - dosagem agronomicamente recomendada) and an overdosage (10x D.A.R.) were tested. Results showed an impact that can be classified as:

D.A.R. Dose	Overdose (10 x D.A.R.)
a) NULL (X)	a) NULL (X)
b) INHIBITORY ( )	b) INHIBITORY ( )
c) STIMULANT ( )	c) STIMULANT ( )

Reference: Certificado Oficial de Análise (Official Analysis Certificate) nº 6942. Série AG – CEPPA – Centro de Pesquisa e Processamento de Alimentos – Universidade Federal do Paraná (Federal University of Paraná).

VIII) Toxicity in soil microorganisms involved in carbon cycle.

The GX 439-0.3% SULFLURAMID 70/30 product was tested in laboratory in order to appraise its toxicity on soil microorganisms involved in the carbon cycle. During periods of 7, 14, and 21 days, in two soil classes (LVe and PVa), the agronomically recommended dosage (D.A.R. - dosagem agronomicamente recomendada) and an overdosage (10x D.A.R.) were tested. Results showed an impact that can be classified as:

D.A.R. Dose	Overdose (10 x D.A.R.)
a) NULL ( )	a) NULL ( )
b) INHIBITORY ( )	b) INHIBITORY ( )
c) STIMULANT (X)	c) STIMULANT (X)

Reference: Certificado Oficial de Análise (Official Analysis Certificate) nº 6934. Série AG – CEPPA – Centro de Pesquisa e Processamento de Alimentos – Universidade Federal do Paraná (Federal University of Paraná).

IX) Toxicity in alga (*Selenastrum capricornutum*)

This study was carried out to determine the toxicity to the green algae *Selenastrum capricornutum* of the product GX 439 (0,3%) (AKI 004-96). Inoculum of  $2,0 \times 10^5$  cells/ml were added to three replicates of the following concentrations: 2,50mg/l; 6,40mg/l; 16,0mg/l; 40,0mg/l; 100mg/l and 250mg/l. Tween 80 (0,025 ml/l) was employed for solubilization of the test substance. After 96 hours exposition, no inhibition of algae culture was observed in all tested concentrations. Therefore, the median effective initial concentration of the product, under the test conditions and based on nominal concentration, was considered greater than 250 mg/l.

Reference: Report # RL 781.069. TECAM – Tecnologia Ambiental.

X) Toxicity evaluation in soil organisms.

The GX 439-0.3% SULFLURAMID 70/30 product presented LC 50 (14 days) > 1,000 mg/kg to the soil organism *Eisenia foetida* in the test conditions. The LC 50 in soil organisms is based on the exposition to a chemical agent that causes lethality to 50% of the organisms in the 14 days of the test period.

Reference: Certificado Oficial de Análise (Official Analysis Certificate) nº 6751. Série AG – CEPPA – Centro de Pesquisa e Processamento de Alimentos – Universidade Federal do Paraná (Federal University of Paraná).

XI) Toxicity evaluation in bees.

The product GX 439-0,3% SULFLURAMIDA 70/30 showed an LD50 - 72 hours > 100 µg/honey-bee to the insect *Apis mellifera* L. at the test condition.

The LD50 to insects, is based on the exposure to a chemical agent which cause lethality of 50% organisms in the 72 hours of period test.

Reference: Certificado Oficial de Análise (Official Analysis Certificate) nº 6926. Série AG – CEPPA – Centro de Pesquisa e Processamento de Alimentos – Universidade Federal do Paraná (Federal University of Paraná).

XII) Acute toxicity evaluation in *Daphnia magna*.

The GX 439-0.3% SULFLURAMID 70/30 product was tested for its toxicity in microcrustacean *Daphnia magna* through laboratory bioassays. The percentage of immobile organisms after a 48 hours exposition to a series of product dilutions and to a control was used in the calculation of the EC (I) 50: 48 h to determine the concentrations:

- a) Minimum, which causes 100% of immobility.
- b) Maximum, which causes no immobility.

The mentioned study was performed in one phase, definitive test.

The results have not allowed the calculation of the EC (I) 50:48 h, there was no immobility of the organisms in the test solutions, including, respectively, the maximum of 100mg/l and the minimum of 0.1 mg/l, as per CETESB methodology, 1991.

Reference: Certificado Oficial de Análise (Official Analysis Certificate) nº 6766. Série AG – CEPPA – Centro de Pesquisa e Processamento de Alimentos – Universidade Federal do Paraná (Federal University of Paraná).

XIII) Acute toxicity evaluation in *Brachydanio rerio*.

The GX 439-0.3% SULFLURAMID 70/30 product was tested for its toxicity on species *Brachydanio rerio* through laboratory bioassays. The percentage of dead organisms after a 96 hours exposition to a series of product dilutions and to a control was used in the calculation of the LC 50:96 hours to determining the concentrations:

- a) minimum, which causes 100% of lethality.
- b) maximum, which causes no lethality.

The mentioned study was performed in one phase, definitive test.

The results obtained have not allowed the calculation of the LC 50:96 h, there was no death of the organisms in the test solutions, including, respectively, the maximum concentration of 100.0 mg/l and minimum of 1.0 mg/l.

Reference: Certificado Oficial de Análise (Official Analysis Certificate) nº 6842. Série AG – CEPPA – Centro de Pesquisa e Processamento de Alimentos – Universidade Federal do Paraná (Federal University of Paraná).

XIV) Acute oral toxicity in birds (*Coturnix coturnix japonica*)

This study was carried out to determine the oral acute toxicity to *Coturnix coturnix japonica* (japanese quail) of the product GX 439 (0,3%) (AKI 004-96) through the determination of the nominal dose that causes 50% of mortality of treated animals (LD50). A limit test was conducted at 2000 mg/kg. Two groups of ten birds each received the maximum dose of the product (2000 mg/kg) and the vehicle (carboxymethylcellulose), respectively, in a single dose by oral gavage. After 14 days of product administration, no mortality was observed among the tested birds. According to the EPA Guidelines, under the test conditions and based on nominal dose, LD50 should be considered greater than 2000 mg/kg.

Reference: Report # RL 334.659. TECAM – Tecnologia Ambiental.

**a) Environmental fate:**

**CHEMICAL / PHYSICAL PROPERTIES:**

- I) Color (EPA § 63-2)  
Off White 5Y/9/2 Munsell Color System (as a Flaky solid)  
Yellow-beige 5Y/8/6 Munsell Color System (as a Waxy solid)  
The raw data is presented in Volume IV of this submission
  
- II) Physical State (EPA § 63-3)  
Waxy Solid (from solidification of a melt) or Flaky Solid (from abrasion of the above waxy solid)  
The raw data is presented in Volume IV of this submission
  
- III) pH (EPA § 63-12)  
pH = 4.43 (1 % dispersion in water)  
The raw data is presented in Volume IV of this submission
  
- IV) Density (EPA § 63-7; OECD Section 1 - 109)  
1.83 g/ml  
The raw data is presented in Volume IV of this submission

- V) Quali-quantitative composition  
 Ref.: Study 1507-QQ-416-04 “Composição quali-quantitativa – Sulfluramida Técnica Dinagro”.  
 Five batches of the test substance were analyzed for active ingredient and impurities. To proceed this test’s, were used the following techniques: Gas Chromatograph and “Karl Fisher”.

Parameter	Sample 1507.1	Sample 1507.2	Sample 1507.3	Sample 1507.4	Sample 1507.5
Sulfluramid (% p/p) (primary isomer)	93,86	93,75	93,27	93,02	93,85
Secondary isomer 1 (% p/p)	4,86	4,81	5,31	5,12	4,94
N,N-dietil perfluorooctano sulfonamide (% p/p)	ND	ND	ND	ND	ND
Moisture (% p/p)	0,13	0,07	0,11	0,09	0,11
<b>Total</b>	<b>98,9</b>	<b>98,6</b>	<b>98,7</b>	<b>98,2</b>	<b>98,9</b>

ND = not detectable

Reference: TASQA - Serviços Analíticos Ltda - Report TSQ - 95821 FQ

- VI) Melting Point (EPA § 63-5; OECD Section 1 - 102)  
 M.P. = 86,1°C (Range: 51°C to 93°C)  
 The raw data is presented in Volume VII of this submission
- VII) Corrosion Characteristics/Oxidizing or Reducing Action (EPA § 63-20 & § 63-14)  
 Visual examination of the immediate package (HDPE) containing the GX 439 sample after two weeks at 48°C – 55°C revealed no corrosive interaction between the GX 439 and its package.  
 See Volume VII of this submission
- VIII) Thermic and in the Air Stability (EPA § 63-13; OECD Section 1 - 113)  
 GX 439 is stable at 48°C – 55°C for a period of 14 days, CIPAC MT 46.  
 The observed melting point of the GX 439 sample from the above storage stability test was found to be 88,04°C which is 2°C higher than observed for the unstressed material. This difference is probably due to sampling as the mixture is not necessarily homogeneous.  
 No exothermic reaction occurred while heating the GX 439 through its melting point.  
 See Volume VII of this submission.
- IX) Vapor Pressure (EPA § 63-9; OECD Section 1 - 104)  
 0,1439 Pa (1,0793 x 10<sup>-3</sup> Torr)  
 The representative raw data is presented in Volume V of this submission

- X) Solubility (EPA § 63-8; OECD Section 1 - 105)  
 Water - 155.7 ppb  
 1-Octanol - 2.712 x 10<sup>8</sup>ppb  
 Acetone - Greater Than 3.91 mg/ml  
 Methanol - Greater Than 13.26 mg/ml  
 The representative raw data is presented in Volume VI of this submission
- XI) Octanol / Water Partition Coefficient (EPA § 63-11; OECD Section 1 - 107)  
 Estimated - 1.74 x 10<sup>6</sup>  
 See Volume VI of this submission
- XII) Dissociation Constant  
 The GX 439 species, through the employed methodology, presented the inflexion point corresponding to its neutralization equivalent, which obtained a value of pKa=9.4.  
 TASQA - Serviços Analíticos Ltda - Report TSQ - 96094 FQ
- XIII) Metal complex formation constant in aqueous mean.  
 The used methodology delivered results for the reference substance (copper-EDTA) coherent with data mentioned in the literature.  
 The tested product, GX 439, has no capacity to form complexes with the metal elements copper, cadmium, and lead.  
 TASQA - Serviços Analíticos Ltda - Report TSQ - 96095 FQ
- XIV) Determination of surface tension in aqueous solution  
Reference Substance  
 Table 1 presents the results obtained in the step of verification of the analytical system, described in item V.C.

Table 1 -Surface Tension of Water at 20°C

Surface Tension	Theoretical value <sup>2</sup> (N/m)
Water (20 °C)	0.07197

Test-Substance

It was used aqueous solution of GX 439. Table 2 shows the results obtained:

Table 2 - Surface Tension of GX 439 at 20°C

Measure	Obtained value (N/m)
1	0,0717
2	0,0713
3	0,0713
Mean	0,0714

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## PERSISTENCE:

### I) Immediate biodegradability

The Sulfluramid HB Técnico product exhibited a value of 42.59% of biodegradation within 28 days, therefore being classified as: "Not easily biodegradable", in the concentrations studied.

Reference: Certificado Oficial de Análise (Official Analysis Certificate) n° 1444. Série AG – CEPPA – Centro de Pesquisa e Processamento de Alimentos – Universidade Federal do Paraná (Federal University of Paraná).

### II) Long term aerobic soil metabolism of $^{14}\text{C}$ -Sulfluramid. (Study XBL n° 93112)

$^{14}\text{C}$ -Sulfluramid was applied to a sandy loam soil of ~75% field moisture capacity at rates of 0.224 (low dose) and 2.24 parts per million ([ppm], high dose), and incubated in the dark at ~30 °C. Samples were harvested at 9 months (i.e., 273 days, low dose) and 12 months (i.e., 365 days, low and high doses) post treatment. Volatiles (including  $^{14}\text{C}$ -carbon dioxide) trapped in potassium hydroxide (KOH) solutions were harvested every month or at each harvesting interval.

Each harvested sample was blended and extracted with acetonitrile / methylene chloride (ACN/MeCl<sub>2</sub>, 3:1). The ACN/MeCl<sub>2</sub> (organosoluble) extract was concentrated for analysis. Most of the applied radioactivity was efficiently and quantitatively partitioned into the organosoluble layer (ca.69%-72%). The radioactivity remaining bound in the post extraction solids (PES) was significant, amounting to ca. 20% - 23%. Acidic volatiles trapped in KOH solutions were considered minor (1.5%-3.0%). Neutral volatiles trapped in polyurethane foam plugs amounted to 5.5%-9.7% of the applied chemical. The overall  $^{14}\text{C}$  recovery was quantitative over the two sampling intervals.

Organoextractables (ACN/MeCl<sub>2</sub>) from soil were analyzed by reversed phase thin-layer chromatography (RP-TLC), as well as normal-phase TLC (NP-TLC) with radiometric detection. The levels of parent chemical were minor, amounting to 1.8%-2.6% of the applied chemical. A number of metabolites were detected (a total of five), designated as Metabolites A through E. Metabolites B and D were the primary contributors to the biodegrade profile, amounting to ca. 44%-55% and ca. 5%-18%, respectively, depending on the treatment rate. Metabolite B was confirmed by chromatography to be desethyl sulfluramid. Metabolite D was characterized as a sulfonic acid metabolite based on similar chromatographic behavior to a reference chemical, perfluorooctane sulfonic acid (potassium salt).

Analysis of the neutral volatile fraction by TLC indicated that the major products contributing to this fraction were parent chemical and Metabolites A and B.

A significant portion of the soil bound residues (PES) could be released by methanol reflux (~9%). Components released from soil by methanol included the parent chemical and Metabolites B, C and D. Reflux with 1 N hydrochloric acid (HCl) released 2.6%-4.2% of the PES. The majority of the remaining radioactivity was released into the humic acid (6.3%-7.1%) and fulvic acid

(2.2%-2.7%) fractions, with 1.0%-1.2% associated with the nonreleasable humin fraction.

Mineralization of sulfluramid and its metabolites (i.e., formation of carbon dioxide) did not occur at any significant level.

III) Mobility of Sulfluramid HB Técnico.

According to the results obtained, Sulfluramid HB Técnico was totally mobile - Class 5 – in Quartz sand, with low content of Organic Matter, and immobile - Class 1 – in Quartz sand (brake), medium dark red latosol and purple latosol, as per IBAMA manual.

Reference: CENA - Centro de Energia Nuclear na Agricultura (E.2 IBAMA test) – Universidade de São Paulo (University of São Paulo).

IV) Adsorption / Desorption of <sup>14</sup>C – GX 071. (Agriseach Project n° 2515)

Although binding of GX071 to test vessels did occur and was significant, it did not affect the calculation of K<sub>d</sub> (adsorption or desorption) since soil bound GX071 was determined by direct combustion of treated soils and the aqueous phases were counted directly. The desorption data also showed that once GX071 was bound to soil and/or glass, it did not desorb back into solution.

The following K<sub>d</sub> values were determined for this study.

SOIL TYPE	ADSORPTION	DESORPTION*
MS-Clay	633	8,155
MD-Sand	118	4,445
MD-Sandy Loam	2296	11,320
CA-Loam	1257	9,775

\* Estimated K<sub>d</sub> based on n = 1 and x/m = K<sub>d</sub>Ce using detection limits.

V) Determination of hydrolysis rate as a function of pH

Tests at Temperatures of 25°C

The results of the tests are in shown Table – 1. The values mentioned refer to GX 439 dosages in the solution.

Table – 1: Hydrolysis of GX 439 – Assay at 25°C

	PH 4,0	pH 7,0	pH 9,0
Co (mg/l) actual initial concentration	7,9	5,6	7,8
Ct (mg/l) Final concentration after “t” days	0,09	0,57	0,91
time (hours)	72	72	72
K <sub>OBS</sub>	0,0622	0,0317	0,0298

Final Results

The final results are displayed in table-3

Table – 3: Hydrolysis of GX 439 – Results at 25°C and room temperature

pH	Temperature	Observed constant (K <sub>OBS</sub> )	Half-life (h)
4,0	25 (°C)	0,062	11
7,0	25 (°C)	0,0317	22
9,0	25 (°C)	0,0298	23



VI) Determination of photolysis in aqueous solution

Reference Substance

The reference substance proposed by EPA<sup>2</sup> protocol was *p-Nitroacetophenone*. When necessary, according to the observed behavior, it is possible to add piridine to work as a half-life regulator. The values found in the *p-Nitroacetophenone* study are displayed in table 1.

Table 1 - Concentrations (ppm) of solutions of *p-Nitroacetophenone* as a function of time and pH

pH	Time (days)											
	0	1	2	3	4	9	11	14	15	16	21	28
pH 4 without light	8,4	8,6	7,9	7,2	7,2	5,4	5,0	4,4	4,3	3,7	3,6	3,6
pH 4 with light	8,4	8,5	7,9	8,2	6,5	5,6	5,9	4,9	4,7	4,1	2,2	0,6
pH 7 without light	8,6	8,9	8,2	6,3	5,0	1,6	---	---	---	---	---	---
pH 7 with light	8,6	8,5	8,2	7,4	7,2	6,5	---	---	---	---	---	---
pH 9 without light	8,2	8,2	8,9	8,1	8,0	8,2	8,4	8,1	8,0	8,0	8,2	8,1
pH 9 with light	8,2	8,0	8,7	8,2	8,3	8,0	8,7	8,2	8,1	8,3	8,2	8,0

Hydrolysis Assay with the GX 439

Table 2 below displays the values of concentration found in the hydrolysis assay (without light) performed in parallel to the photolysis assay (with light).

Table 2 - Concentrations (ppm) of incubated solutions in lack of light (hydrolysis).

pH	Time (days)	
	0	3
pH 4 without light	7,9	0,09
pH 7 without light	5,6	0,57
pH 9 without light	7,8	0,91

Photolysis Assay with the GX 439

Table 3 below displays the values of concentration found in the photolysis assay (with light). In Annex 2, there is a chart concerning the assays of hydrolysis and photolysis with the sample.

Table 3 - Concentrations (ppm) of incubated solutions in the presence of light (photolysis).

pH	Time (days)	
	0	3
pH 4 with light	7,9	0,9
pH 7 with light	5,6	0,4
pH 9 with light	7,8	1,86

Calculation of photolytic half-life of the reference substance

pH	Temperature (°C)	K <sub>OBS</sub> (mg/l)	Half-life (hours)
4,0	25	0,0039	176
7,0	25	0,0013	535
9,0	25	0,0001	> 1 year

Calculation of hydrolytic half-life of GX 439

pH	Temperature (°C)	K <sub>OBS</sub> (mg/l)	Half-life (hours)
4,0	25	0,0622	11
7,0	25	0,0317	22
9,0	25	0,0298	23

Calculation of photolytic half-life of GX 439

pH	Temperature (°C)	K <sub>OBS</sub> (mg/l)	Half-life (hours)
4,0	25	0	> 1 year
7,0	25	0,0004	1618
9,0	25	0	> 1 year

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**HOW ARE CHEMICAL / PHYSICAL PROPERTIES AND PERSISTENCE LINKED TO ENVIRONMENTAL TRANSPORT, TRANSFER WITHIN AND BETWEEN ENVIRONMENTAL COMPARTMENTS, DEGRADATION AND TRANSFORMATION TO OTHER CHEMICALS?**

Sulfluramid degrades at a rapid rate in soil incubated under aerobic conditions. Degradation proceeds initially by oxidative deethylation to form desethyl sulfluramid and other metabolites. Decomposition is accompanied by the formation of soil bound residues, including the parent compound and its metabolites, as well as other materials incorporated into humic and fulvic acids. Loss of the parent chemical, as well as Metabolites A and B, can also occur through volatilization albeit at minor amounts.

The sulfluramid used in the form of ant bait virtually presents no risks to human health and to environment due to the following points:

- The product is specific to the plague it is intended for.
- The amount of active principle used for manufacturing the ant baits is very reduced (3 g/kg or 0.3%) and the amount of baits used by m<sup>2</sup> of nest area is also very small (6 - 10 g/m<sup>2</sup>).
- Its presentation form – Granular Baits (Pellets) – and the kind of application – straight from the package, without manual contact – virtually prevents all risks to man and environment upon handling and application.
- The application of the product is performed in a local manner, next to the active holes of the anthill, with the ants quickly carrying the baits into the nest.

- The sulfluramid is immobile in many kinds of soil, has very low solubility in water and is strongly adsorbed or bounded to organic matter present in the soil, as well as its main metabolite. The low water solubility of sulfluramid and its metabolites and its high capacity to be bounded to the organic matter in the soil virtually prevents its rollover to the water bodies and the environment.

### **BIO-CONCENTRATION OR BIO-ACCUMULATION FACTOR, BASED ON MEASURED VALUES**

#### I) Bioconcentration in fish (*Brachydanio rerio*).

This study was carried out to determine the accumulation potential of the product TECHNICAL SULFLURAMIDE HB (AKI 001-95) in the tissues of *Brachydanio rerio*. One hundred animals were exposed to the average concentration of 8 µg/L of test substance for 14 days in semi-static system (uptake phase). Samples of water and fishes were periodically collected during this period (total of six samples). Then, animals were kept in clean water for seven days (clearance phase) and four samples were collected. According to the bioconcentration model adopted, the rate constant of uptake (k1) was calculated in 53,7 L/g/h, the rate of clearance (k2) in 0,05/h and the Bioconcentration Factor (BCF) in 1131.

Reference: Report # RL 833.434. TECAM – Tecnologia Ambiental.

#### II) GX 071 – Tissue Kinetics in rats after dietary exposure. (Study n° 055)

Sulfluramid (N-ethylperfluorooctane sulfonamide) is the active ingredient of an insecticide recently introduced for residential control of household ants and roaches. Sulfluramid is currently being studied for widespread control of the red imported fire ant. Sixty male, Sprague-Dawley rats were fed either ground rodent feed non-spiked or spiked with 75 mg sulfluramid/kg feed for 56 days and then fed the non-spiked, diet for an additional 35 days. Nine solid tissue samples and one blood sample were collected and analyzed periodically during the study. Rats consumed a mean of  $5.7 \pm 0.6$  mg sulfluramid per kg/day throughout the 56-day dosing period. No overt clinical signs of toxicity were observed during the study. There was a significant decrease (7%) in body weight gained in the treated group compared to the controls, but no significant difference in feed consumption between the two groups. There were no detectable levels (minimum detectable level = 0.1 µg/g tissue) of sulfluramida present in any tissue samples during the study. By day 7 (the initial sampling time) of the treatment period, desethylsulfluramid, the major metabolite of sulfluramid, had reached a steady-state concentration (C<sub>ss</sub>) in all tissues and blood. The highest concentrations (mean ± SD) of desethylsulfluramid were detected in the liver ( $29.1 \pm 0.8$  µg/g), lung ( $30.1 \pm 2.4$  µg/g) and fat ( $23.4 \pm 4.3$  µg/g). The lowest concentrations were in skeletal muscle ( $7.1 \pm 0.5$  µg/g) and testes ( $5.9 \pm 0.3$  µg/g). Desethylsulfluramid exhibited first order elimination kinetics. The blood half-life of 8.1 days was statistically greater than the mean

half-life for solid tissues ( $2.3 \pm 0.8$  days). Neither sulfluramid nor desethylsulfluramid accumulated in the tissues of the rat.

III) Metabolism and disposition of sulfluramid, a unique polyfluorinated insecticide, in the rat.

The objectives of this study were to characterize the absorption, distribution and elimination of sulfluramid (N-ethyl perfluorooctane sulfonamide) and its major metabolite, perfluorooctane sulfonamide (DESFA), in order to assess the effect of dosage vehicle on their pharmacokinetics. In trial 1, male and female Sprague-Dawley rats (170-240 g) were housed in Roth-type metabolism cages. Each rat received 50 mg/kg sulfluramid po, which contained 10  $\mu$ Cl of [ $^{14}$ C] sulfluramid. Feces, urine and expired air samples were collected for 72 hours post dosing. Tissue samples also were collected at 72 hours and  $^{14}$ C distribution determined. Eighty percent of the radiolabel was eliminated within 72 hours, with the largest quantities of  $^{14}$ C recovered in expired air (66%) and feces (25%). Less radiolabel was recovered in urine (8%), and even smaller amounts in tissues (5%). The highest tissue concentrations of  $^{14}$ C were found in liver, kidneys and adrenals; with significantly more radiolabel in the kidneys, gonads and adrenals of the females than males. Male Sprague-Dawley rats (250-275 g) were used in Trials 2 and 3. In Trial 2, rats with a carotid artery cannula were given 50 mg/kg sulfluramid in a po bolus of polyethylene glycol 400 (PEG) or corn oil and blood samples were collected for 96 hours. In Trial 3, noncannulated rats were given 50 mg/kg sulfluramid po in PEG or corn oil and blood samples were collected from the caudal artery for 14 days. Blood samples were analysed for sulfluramid and DESFA by gas chromatography. Pharmacokinetics analyses indicate that sulfluramid is absorbed slowly from the gastrointestinal tract but is converted to DESFA quickly, and that DESFA has an extremely long half-life compared to that of sulfluramid. Corn oil appeared to increase and prolong the absorption of sulfluramid from the gastrointestinal tract, but the effects were not dramatic.

Reference: Randall, O. Maning, James V. Bruckner, Michael E. Mispagel and John M. Bowen "Metabolism and disposition of sulfluramid, a unique polyfluorinated insecticide, in the rat." College of Veterinary Medicine, University of Georgia, 1990.

IV) Distribution and tissue elimination in rats during and after prolonged dietary exposure to a highly fluorinated sulfonamide pesticide.

Rats were fed either nonfortified, ground rodent feed or feed fortified with 75 mg of sulfluramid/kg of feed for 56 days and then were fed the nonfortified diet for an additional 35 days. Samples from nine tissues and blood samples were collected and analyzed by gas chromatography. No overt clinical signs of toxicity were observed during the study except for a significant decrease in body weight gained in the treated group compared to the controls. This difference appeared to result from a significant reduction in rate of weight gain during the first 2 weeks of treatment. Diet consumption was not significantly different for treated and control groups. There were no detectable levels of sulfluramid

present in tissue or blood samples during the study, but its metabolite, deethylsulfluramid, was present, and changes in its concentration were associated with first-order elimination kinetics. The blood half-life of 10.8 days was greater than those for the solid tissues. The half-life for fat was 1.1 days. Though highly lipid soluble, neither sulfluramid nor deethylsulfluramid accumulated in the tissues of the rats during the 56-day dosing period.

Reference: Mark R. Grossman, Michael E. Mispagel and John M. Bowen "Distribution and tissue elimination in rats during and after prolonged dietary exposure to a highly fluorinated sulfonamide pesticide." College of Veterinary Medicine, University of Georgia, Published at J. Agric. Food Chem., Vol. 40, n° 12, p. 2505-2509, 1992.

## MONITORING DATA

- I) Assessment of environmental risk of ant baits (formicide) based of sulfluramid in forest area.

The environment monitoring project of SULFLURAMID was conducted in a 30 ha forest area with eucalyptus, where ant baits containing SULFLURAMID were applied. An area of native brake of 500 m<sup>2</sup> was maintained without the ant baits to serve as control treatment. During 1 year, samples of soils, water, fishes and wild rats were collected to determine the concentration of the residues of SULFLURAMID and its main metabolite, the perfluorooctane sulfonamide (DESFA). Daily observation was also done in the project site for the possible identification of any other dead animal. The analysis of residues to all matrixes: water, soils, fishes e wild rats (blood and fats) did not presented any level of residues both for SULFLURAMID and its metabolite DESFA. The results found, aside the lack of any mortality of fishes and animals in the site, revealed that there was no negative effect to the local fauna caused by the use, in commercial dosage, of the ant baits.

Reference: "Avaliação de risco ambiental de iscas formicidas a base de sulfluramida em área florestal." (Assessment of environmental risk sulfluramid-based ant baits in forest area). Proj 01/97 – Bioagri Laboratórios Ltda.

- II) Eficiência agrônômica do produto Mirex-S Max no controle de *Atta capiguara* Gonçalves, 1944 (Hymenoptera: Formicidae) e análise de resíduos de sulfluramida em capim e solo. (Agronomic efficiency of the Mirex-S Max product in the control of *Atta capiguara* Gonçalves, 1944 (Hymenoptera: Formicidae) and analysis of the residues of sulfluramid in grass and soil).

The present study, rolled out in areas of pasture of the Santo Antônio farm, municipality of Pirajuí, S.P., in July, 12, 1997, intended to assess the agronomic efficiency and the residue levels of Sulfluramid, in samples of grass and soil collected around and over the nests of *Atta capiguara*, treated with the commercial product Mirex-S Max, a granulated formicide, formulated with attractive substrate (orange pulp), added with 0.3% of active principle

Sulfluramid. BATISTA, 1985, performed a similar study of residues in soil and pastures, but with colonies of *Atta capiguara* treated with ant baits based on dodecachlor, and obtained, as a result, residues of that active principle in all soil samples, which were not detected in the grass samples. For this experiment, 10 nests were used of *Atta capiguara*, which received application of the Mirex-S Max commercial product at the recommended dosage for control (10 g/m); from these, 05 were chosen, through raffle, to compose the samples of soil and grass to be analyzed, with one nest being the testimony. For the assessment, the collection of samples was performed on the 20<sup>th</sup> and 200<sup>th</sup> days after product application. The samples of soil were collected in 10 points of area of each nest, in a depth of 30 cm, which were mixed in plastic bags for later removal of around 1 kg of soil, making up a composed sample of each nest. The samples of grass were collected over 5 of 10 sampled points, which were mixed in plastic bags to, similarly, to compose 1 kg of composed sample of the area of each nest. Those samples were properly packed and frozen at -18°C, in a freezer, remaining at this temperature until immediately prior the analysis, run at Laboratório TASQA Serviços Analíticos Ltda., where the whole study of the residue was performed using validated methodology for this active principle. In the analysis, the Sulfluramid was extracted through organic solvent and recuperation studies were performed at the level of concentration of 0.1 mg/kg, and the obtained results were of 112.0% to the grass and 85.9% to the soil. The limit of detection used was 0.1 mg/kg and the quantification limit was 0.03 mg/kg, to which were found no residues of Sulfluramid in any of the grass and soil samples, both in the samples collected on the 20<sup>th</sup> and on the 200<sup>th</sup> day after product application. The mortality of the nests was assessed 200 days after product application, and an agronomic efficiency of 90% was obtained.

Reference: Ramos, Vânia M.; Forti, Luiz C.; Andrade, Ana Paula P. "Eficiência agrônômica do produto Mirex-S Max no controle de *Atta capiguara* Gonçalves, 1944 (Hymenoptera: Formicidae) e análise de resíduos de sulfluramida em capim e solo. (Agronomic efficiency of the Mirex-S Max product in the control of *Atta capiguara* Gonçalves, 1944 (Hymenoptera: Formicidae) and analysis of the residues of sulfluramid in grass and soil). *Naturalia*, V.24, p. 283-285, 1999.

## EXPOSURE IN LOCAL AREAS

Due to its physical-chemical properties, such as very low solubility in water, little or no mobility in many kinds of soil, high capacity of adsorption to organic matter and, mainly due to its use as ant bait, product presentation (solid formulation for prompt use, in the form of pellets), mode of application (near active holes of the nest, with the ants quickly carrying the baits into the nest), and due to it being a specific product to the plague it is intended for (formicide for the control of leaf-cutting ants), we can state that the occurrence of transportation of sulfluramid to long distances in the environment and its bioaccumulation is unlikely.

**NATIONAL AND INTERNATIONAL RISK EVALUATIONS, ASSESSMENTS OR  
PROFILES AND LABELLING INFORMATION AND HAZARD  
CLASSIFICATIONS, AS AVAILABLE.**

Sulfluramid fits into toxicological class IV – slightly toxic, and is rated environmental hazard potential II – very dangerous.

Sulfluramid-based baits (0.3%) fit into toxicological class IV – slightly toxic, and are rated environmental hazard potential III – dangerous.