Discovery and Development of a New Insecticide 'amidoflumet (Panduck®)' with High Miticidal Activity against House Dust Mites

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Amidoflumet is a new trifluoromethanesulfonanilide compound with high house dust miticidal activity which was discovered by Sumitomo Chemical, and was registered in Japan in 2004. House dust mites and their products are known to be major household allergens to children and the elderly, and they cause asthma and atopic dermatitis. Amidoflumet shows high lethal activity against common house dust mites. In particular amidoflumet has excellent activity against predatory cheyletid mites, which often cause biting injuries to humans. These efficacies and its excellent safety to mammals can provide us with an important tool for controlling various house dust mites. This paper describes the discovery story, miticidal efficacies in various formulations, a method of synthesis and safety evaluations of amidoflumet.

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Introduction

Various types of mites inhabit the typical home environment, but among these, house dust mites that feed on human dandruff and food residues are found in the largest numbers. Their corpses and feces cause allergies.[1)] Therefore, control of house dust mites is an important problem in terms of measures for allergic disorders, which have been increasing in children and elderly people in recent years, particularly, asthma and atopic dermatitis. Other known mites include mold mites (*Tyrophagus putrescentiae*), which occur in large numbers in stored food products and *tatami* mats (Japanese straw floor covering), as well as cheyletid mites, which are predatory mites. Pyrethroid insecticides have been used for the most typical house dust mite control agents, but their effect has not always been satisfactory on the various types of mites that inhabit households. In particular, there have been no known compound that exhibit a high level of effect on the cheyletid mites that attack humans.

At Sumitomo Chemical Co., Ltd., we have been searching for a new house dust mite control agent that is highly effective over a wider spectrum than conventional miticides for indoor use, and we have discovered and developed amidoflumet (Panduck®), which is a new compound that exhibits activity not seen in existing miticides. Amidoflumet has a higher lethal efficacy for the house dust mites that inhabit households in general than conventional agents and is faster acting, and a salient feature is its high lethal efficacy against cheyletid mites, which have been difficult to control with the agents used up to now. Amidoflumet has superior stability and can be adapted to various types of formulations. In addition, the products that contain amidoflumet for mite control have a level of safety that is the same or more than the products actually used up to now.

In this paper, we will report on the history of the discovery of amidoflumet, the efficacy of various preparations on house dust mites, physical and chemical properties, manufacturing methods and safety evaluations. History of the Discovery.

1. Research Background

The corpses and excretions of house dust mites such as the American house dust mite (*Dermatophagoides farinae*) and the European house dust mite (*D.*

pteronyssinus), which are the main household dust mites, are thought of as being the main allergens causing asthma and dermatitis in children and elderly people. Furthermore, there is a strong desire to eliminate allergens from households to avoid the onset of these. Pyrethroid agents such as phenothrin, and ester compounds such as phenyl salicylate (1) and benzyl benzoate (2), which are shown in **Fig. 1**, are used as agents for preventing household dust mites.

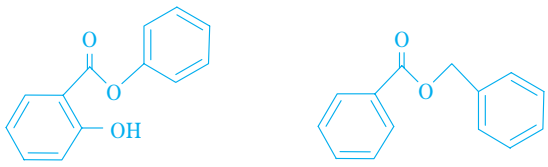

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Fig. 1 Typical active ingredients for house dust mites

However, there are problems in that the efficacy of these agents is insufficient for house dust mite and mold mite control, and they exhibit almost no effect on cheyletid mites which cause a great deal of damage by biting humans. In addition, these cheyletid mites are pests that are difficult to remove, and it is known that other miticides cannot remove them sufficiently.[2] The authors started on investigative research to discover a household dust mite controlling agent that had a high level of efficacy for these cheyletid mites. Moreover, we focused on not only house dust mites, but also mold mites and cheyletid mites.

2. Discovery of Insecticidal Trifluoromethanesulfonanilide Compounds

Previously, the authors had discovered that the series of trifluoromethanesulfonanilide compounds (3) shown in **Fig. 2** exhibited a high level of lethal activity for houseflies and cockroaches.[3]

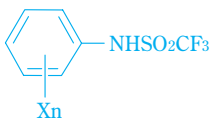

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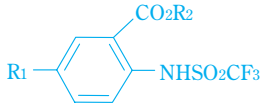
Fig. 2 Insecticidal trifluoromethanesulfonanilides

3. Discovery of Amidoflumet (Panduck®)

Therefore, we focused on the miticidal activity of this group of compounds and synthesized a series of trifluoromethanesulfonanilide compounds (4–15) containing alkoxycarbonyl groups at the 2-position of a trifluoromethanesulfonanilide shown in **Fig. 3** from structural similarities with phenyl salicylate (1) and benzyl benzoate (2).



4 : R_1=Cl, R_2=Me
5 : R_1=Cl, R_2=Et
6 : R_1=Cl, R_2=iPr
7 : R_1=Cl, R_2=tBu
8 : R_1=Cl, R_2=Ph
9 : R_1=Br, R_2=Me
10 : R_1=I, R_2=Me
11 : R_1=Me, R_2=Me
12 : R_1=NO_2, R_2=Me
13 : R_1=OMe, R_2=Me
14 : R_1=CF_3, R_2=Me
15 : R_1=H, R_2=Me

Fig. 3 Synthetic method of 2-alkoxycarbonyltrifluoromethanesulfonanilides

The test results are shown in **Table 1** and **2**.

As a result, it was clear that compounds with halogen atom substitutions at the 4-position on the benzene ring exhibited a high lethal activity for both American house dust mites (*Df*) and mold mites (*Tp*). On the other hand, compounds 12 and 14 with nitro group and trifluoromethyl group substitutions, which are both electron withdrawing groups, showed insufficient activity.

In addition, compounds 11 and 13 with a methyl group and a methoxy group substitutions, which are sterically about the same size as a chlorine atom, only

Table 1 Miticidal activity of 2-alkoxycarbonyltrifluoromethanesulfonanilides against *D. farinae (Df) and T. putrescentiae (Tp)*

Compound			Dose (mg/m²) – Activity (%)	
No.	R_1	R_2	*Df* 8 (mg/m²)	*Tp* 80 (mg/m²)
4	Cl	Me	+ + +	+ + +
5	Cl	Et	+ + +	+ + +
6	Cl	iPr	+ + +	+ + +
7	Cl	tBu	+ + +	+ + +
8	Cl	Ph	+ + +*	–
9	Br	Me	+ + +	+ + +
10	I	Me	+ + +	+ + +
11	Me	Me	+ + +*	–
12	NO_2	Me	–	+ + +
13	OMe	Me	+*	–
14	CF_3	Me	–	+ + +
15	H	Me	+ + +*	–

+ + + : 100% mortality, + + : > 90% mortality, + : 70 – 90% mortality,
– : Almost the same as untreated sample
* : 80 (mg/m²)

Table 2 Miticidal activity of 2-alkoxycarbonyltrifluoromethanesulfonanilides against *C. moorei (Cm)*

Compound			Dose (mg/m^2) – Activity (%)
No.	R_1	R_2	*Cm* 80 (mg/m^2)
4	Cl	Me	+ + +
5	Cl	Et	+ + +
6	Cl	iPr	+ + +
7	Cl	tBu	+ + +
9	Br	Me	+ + +
10	I	Me	+ + +

+ + + : 100% mortality, + + : > 90% mortality, + : 70 – 90% mortality, – : Almost the same as untreated sample

Table 3 Physical and chemical property of amidoflumet

Molecular formula	$C_9H_7ClF_3NO_4S$
Molecular weight	317.67
Appearance	Slightly yellow or colorless crystalline solid
Melting point	81 ~ 85°C
Vapor pressure	ca. 1.51×10^{-1} Pa (Gas saturation method)
Acid dissociation constant	pKa = ca.3.8
Distribution coefficient	logP = 2.13 (pH5, 24°C) (Ambient shake flask method)
	logP = 4.13 (pH1, 24°C) (do.)
	logP = –0.28 (pH9, 24°C) (do.)
Solubility	Soluble in following solvents : Acetonitrile, N,N-Dimethylformamide, Acetone, Methanol, Ethanol
Thermal analysis	Endothermy observed at ca.82°C
	Weight loss by vaporization began at around 80°C

exhibited a low level of activity.

Next, when we focused on alkoxycarbonyl groups at the 2-position on the benzene ring, and compounds where R_2 was a lower alkyl group with one to three carbon atoms exhibited a high level of lethal activity against both American house dust mites and mold mites. On the other hand, there was a significant decrease in the activity with the phenyl ester (8), which is sterically larger than a lower alkyl group.

Furthermore, the compounds 4–7, 9 and 10, which exhibited a high level of activity for American house dust mites and mold mites, also showed the same high level of activity for *Chelacaropsis moorei* (*Cm*). From these results, it was clear that a number of one to three carbon atoms was suitable for R_2.

From the activity on some species of house dust mites above and from the activity in various formulations, we selected the compound (4) shown in **Fig. 4** as the representative compound, and this led to the new house dust mite control agent amidoflumet (Panduck®).[4)]

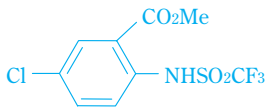


Fig. 4 Structure of amidoflumet

Physical Properties and Stability

1. Physical and Chemical Properties

The physical and chemical properties of amidoflumet are given in **Table 3**. Amidoflumet is a pale yellow to white crystalline powder. It is easily dissolved in polar solvents such as N,N-dimethylformamide, acetonitrile and methanol, but it dissolves poorly in water. The melting point is around 82°C, and it does not decompose when melted. The dissociation constant pKa is approximately 3.8, and as the pH becomes lower the proportion of the nonionic form increases, so the solubility in water becomes lower. For solutions at various pH (1, 5 and 9), the 1-octanol layer partition increased as the pH became lower. In differential thermal analysis, endothermy and weight loss because of melting around 80°C were observed.

2. Stability

Amidoflumet was stable even when stored for 36 months at 25°C and humidity of 60% and for six months at 40°C and humidity of 75%.

Table 4 Stability of amidoflumet

Storage conditions	25°C 60%RH In the dark	40°C 75%RH In the dark
Storage period	36 months	6 months
Container	Polyethylene bag	Polyethylene bag
Content	Stable	Stable

Storage conditions	50°C In the dark	25°C 100%RH In the dark	25°C 1000 lux
Storage period	3 months	3 months	50 days
Container	Glass vial (capped)	Glass vial (open)	Petridish covered with PVDC* film
Content	Stable	Stable	Stable

* : Poly vinylidene chloride

In addition no affect of temperature, humidity or exposure to sunlight was seen. It was stable when stored for three years at room temperature (**Table 4**), and was generally stable in various types of common solvents, but an ester exchange reaction may occur in ethanol (**Table 5**). Next, we examined the stability in solid carriers. It was stable in azodicarbonamide which is added to foaming agents such as heat vaporizing agents, and even in talc, silica and other carriers it generally exhibited good stability (**Table 6**).

Table 5 Stability of amidoflumet in various solvents as 1%w/v solution

Solvent	Recovery rate (%)*
Methanol	99
Ethanol	80
2-Propanol	100
Methylene chloride	100

* : Recovery rate of the sample which was stored at –5°C expressed as 100%
Storage condition : glass ampule, 60°C · 1 month

Table 6 Stability of amidoflumet in various carriers as 1%w/w powder

Carrier	Recovery rate (%)*
Azodicarbonamide	100
Talc	96
Silica	98

* : Recovery rate of the sample which was stored at –5°C expressed as 100%
Storage condition : glass ampule, 60°C · 1 month

3. Effects on Metals

Heat vaporizing agents, total release aerosols and other dust mite extermination uses that can be conceived of for amidoflumet are preparations that treat the entire space of rooms. With preparations of this type, the effective ingredients that are released into the air may adhere to metal used in articles such as furniture. Therefore, we examined the effects of amidoflumet on various kinds of metal. Various metal powders were put onto filter paper impregnated with a prescribed amount of amidoflumet and spread thinly. The paper was folded in half with this surface on the inside, sealed, and the effects on the metal were observed after the sample was stored at prescribed conditions. As a result, no effects from being in contact with the amidoflumet were observed in any of the metals (**Table 7**).

Table 7 Contact compatibility of amidoflumet with metal powder

Metal powder	Appearance after storage*
Iron	NA
Lead	NA
Copper	NA
Tin	NA
Zinc	NA
Aluminium	NA
Blonz	NA

Test sample : Amidoflumet 1.53mg + metal powder 0.03mg/cm^2 (filter paper)
* : Packaged in aluminium-laminated polyethylene bag at 60°C for 1 month
** : NA : Not affected

Efficacy and Formulation

1. Basic Activity

The lethal activity of amidoflumet on household dust mites using the clip method is shown in **Table 8**. In this method, a bag is made out of filter paper that is treated with the agent, mites are closed inside, and the lethal activity is examined after one day. The mites always come into contact with the agent, and there is no worry about their escaping during the treatment. Therefore, it is a suitable method for measuring the basic activity of agents. Amidoflumet exhibited a high mortality of 90% or more on American house dust mites in all treatments, and it exhibited activity superior to phenyl salicylate and benzyl benzoate. On the other hand, the mortality of amidoflumet for mold mites was 100% at 500 mg/m^2, but at 100 mg/m^2, it dropped to 50%. There was deterioration in the activity for mold mites, but the activity was about the same as that for phenyl salicylate and benzyl benzoate. Furthermore, while amidoflumet exhibited a 100% mortality for *Chelacaropsis moorei* at 500 mg/m^2, phenyl salicylate and benzyl benzoate exhibited absolutely no lethal

Table 8 Lethal activity of amidoflumet against house dust mites at 500, 100, and 20mg/m^2 by filter paper contact method

Compound	*D. farinae*			*T. putrescentiae*			*C. moorei*		
	500	100	20	500	100	20	500	100	20
Amidoflumet	100	99	94	100	47	1	100	63	18
Phenyl salicylate	100	45	28	100	51	6	0	–	–
Benzyl benzoate	100	19	6	100	69	1	0	–	–

activity at 500 mg/m^2. From these results, amidoflumet exhibited the greatest lethal activity for American house dust mites among the three types of house dust mites, and it was shown that there was a high level of lethal activity for *Chelacaropsis moorei*, which is difficult to control. In particular, the aspect of effectiveness for the latter may be thought of as a very salient characteristic for amidoflumet as a house dust mite agent.

The quickness of the action is an extremely important factor in the agent for minimizing the biting damage from house dust mites. In addition, with house dust mite agents, there is a possibility that the mites may temporarily escape the treatment depending on the repellency of the agent, but when the effect disappears they return to the original location. An agent that acts rapidly and has no repellency is desirable. Therefore, we investigated the quickness of the amidoflumet activity and its repellency, using American house dust mites and a filter paper contact method (the treatment being 800 mg/m^2). The results were that 5 minutes after the amidoflumet treatment, the activity of all individuals ceased (**Fig. 5**). On the other hand, with phenyl salicylate and benzyl benzoate, 50% of more of the individuals were active 5 minutes after treatment, but all activity of the individuals ceased with both agents after 30 minutes. There was absolutely no repellency exhibited with amidoflumet at 800 mg/m^2, while phenyl salicylate and benzyl benzoate exhibited some repellency (**Fig. 6**). From these results, it is clear that amidoflumet acts on house dust mites extremely rapidly and exhibits a high level of lethal activity.

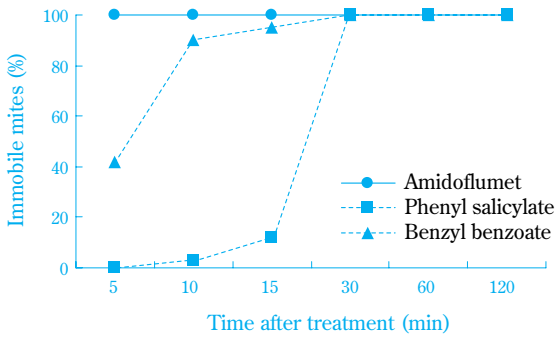


Fig. 5 Action speed of amidoflumet against *D. farinae* by paper contact method.
Application dose of each compound was 800 mg/m^2

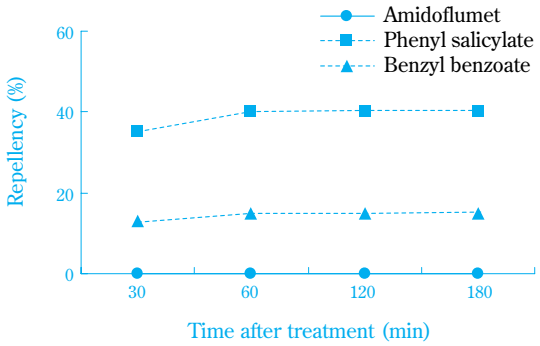


Fig. 6 Repellency of amidoflumet against *D. farinae* by paper contact method.
Application dose of each compound was 800 mg/m^2

2. Sheet Preparation

Sheet preparations, where sheets treated with the agent are laid underneath the carpets or tatami mats that house dust mites inhabit, are an extremely labor-saving control method for which the efficacy can be expected to continue over a long period of time. Therefore, we prepared a sheet preparation where the agent was applied to kraft paper, which is the same as commercial sheet preparations, and laid carpet over this. American house dust mites were released onto the carpet, and the change inpopulation was examined. As a result, there was an extremely high level of efficacy which exhibited an inhibitory rate of reproduction of 98.5% after one week and 100% after both three and six weeks with a 500 mg treatment of amidoflumet per square meter of carpet. While it was a simple test, it showed the efficacy of the sheet preparation (**Fig. 7**).

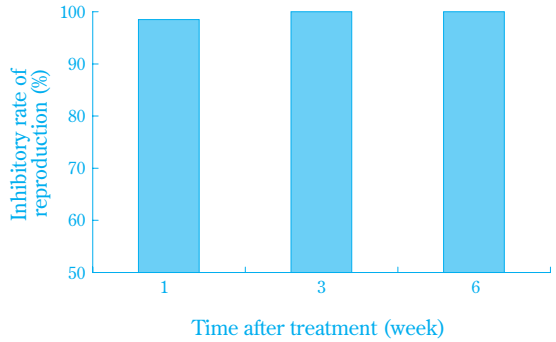


Fig. 7 Effect of amidoflumet on *D. farinae* population on carpet by sheet method.
Application dose of amidoflumet was 500 mg/m^2

3. Aerosol Preparation

Aerosol preparations are preparations for which simple use with rapid effects can be expected. Aerosol preparations for house dust mites are already on the

market, and they are devised so that treatment can be done by inserting the tip of a nozzle into a tatami mat. We compared the efficacy of an aerosol preparation of amidoflumet on American house dust mites, mold mites and *Chelacaropsis moorei* with phenothrin, which is a pyrethroid insecticide (**Fig. 8**). The test aerosol preparation mixed each active ingredient with no. 1 kerosene and filled in a dimethyl either propellant, and it was prepared such that the active ingredient was completely dissolved. Each square meter of floor surface was treated with 62 mg of amidoflumet, and the mortality of the American house dust mites, mold mites and *Chelacaropsis moorei* after one day was 100%, 100% and 96.7% respectively, which it showed an efficacy superior to that of a 65 mg treatment with phenothrin. Moreover, amidoflumet exhibited almost no repellency for any of the mites, but repellency was seen with phenothrin.

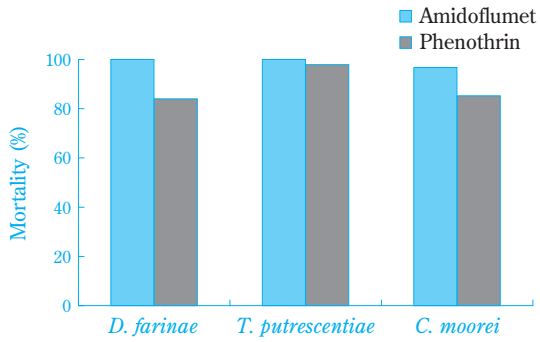


Fig. 8 Efficacy of amidoflumet against house dust mites on glass dish by aerosol spray method. Application doses of amidoflumet and phenothrin were 62 and 65 mg/m^2, respectively

4. Heated Vaporization Preparation

Preparations that treat a large space by releasing the active ingredient all at once, and control harmful insects inhabiting a place include smoking agents fumigants, heated vaporization agents and total release aerosol (TRA) agents. The former two are preparations that release the active ingredient as a gas generated by combustion heat, chemical reaction heat or heating. The latter are preparations that release all of the stock solution that contains the propellant gas and active ingredient all at once. According to thermal analysis, amidoflumet begins losing weight starting in the neighborhood of 80°C, and almost all of it is vaporized by 180°C. Because of this characteristic of efficient volatilization at a comparatively low temperature range, we thought that amidoflumet was more suitable for a heated vaporization preparation that volatilizes the active ingredient at a lower temperature than smoking agents, where the inside of the preparation reaches extremely high temperatures accompanying the combustion reaction.

Therefore, we prepared a heated vaporization preparation of amidoflumet, and carried out semi- field tests with commercial products as the control.

A granulated powder where 0.5g of bulk amidoflumet was made to impregnate a sprayed powder mixed with azodicarbonamide was prepared. The heated vaporization preparation for testing was prepared by placing a heating material (calcium oxide) with this granulated powder on the outside in a metal container. The sprayed powder was heated by a chemical reaction between the heating material and water in a large chamber (length × width × height : 4 × 3 × 2.3 m = 28 m^3), and when the efficacy against American house dust mites on the carpet was examined, about the same effect as the commercial product was obtained (**Fig. 9**), and the effectiveness of amidoflumet in a heated vaporization preparation was confirmed.

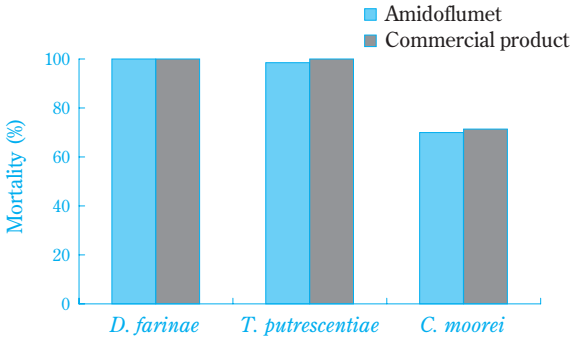


Fig. 9 Efficacy of amidoflumet against house dust mites on a glass dish by fumigation. A commercial product contained phenothrin and methoxadiazone

5. Total Release Aerosol Preparation (TRA)

We investigated the practical efficacy of amidoflumet in a total release aerosol preparation which is a preparation for treating large spaces in the same manner as the heated vaporization preparation. The total release aerosol preparation for testing was prepared by dissolving 0.5g of bulk amidoflumet in a mixed liquid of isopropyl alcohol and no. 1 kerosene and using dimethyl either as the propellant. A glass petri dish containing American house dust mites, mold mites and *Chelac-*

aropsis moorei and a carpet inoculated with American house dust mites were placed in a large chamber and the test aerosol preparation was totally released. The mites in the glass petri dish were examined one day after treatment and those in the carpet four days after treatment.

As a result, the amidoflumet exhibited a high mortality of 100% for the American house dust mites and mold mites and 97% for *Chelacaropsis moorei* in the glass petri dish (**Fig. 10**). In addition, it exhibited a high mortality of 98% for the American house dust mites in the carpet. This is about the same efficacy as the commercial preparation (**Fig. 11**), and we were able to confirm the effectiveness of the total release aerosol preparation.

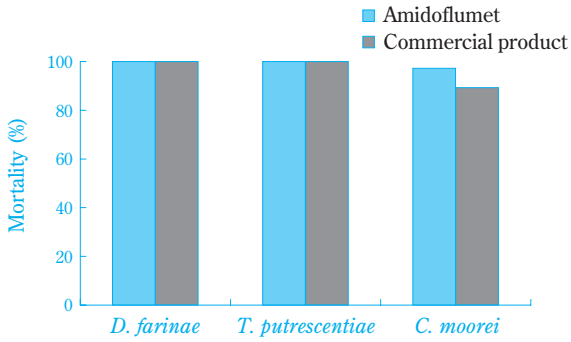


Fig. 10 Efficacy of amidoflumet against house dust mites on a glass dish by total release aerosol. A commercial product contained phenothrin and methoxadiazone

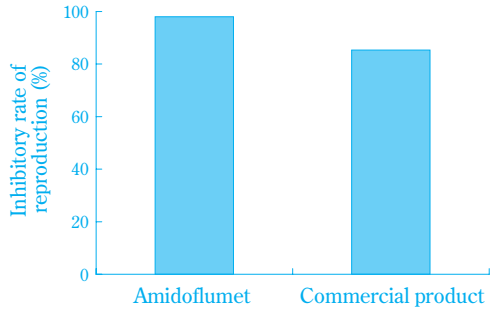


Fig. 11 Efficacy of amidoflumet against *D. farinae* on carpet by total release aerosol. A commercial product contained phenothrin and methoxadiazone

As above, amidoflumet exhibited a rapid action and high level of lethal activity for American house dust mites and mold mites, which are typical house dust mites, and it was clear that it also had a high level of lethal activity for *Chelacaropsis moorei*, which has been difficult to eliminate with existing compounds up to this point. Furthermore, since amidoflumet can be prepared in many types of preparations such as sheet preparations, heated vaporization preparations and total release aerosol preparations, it is expected to contribute to the elimination of house dust mites.

Manufacturing Methods

Amidoflumet is a trifluoromethanesulfonanilide compound, and as is shown in **Fig. 12**, it can be manufactured by a reaction of methyl 5-chloro-anthranilate and trifluoromethanesulfonic anhydride.

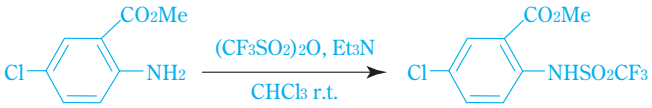


Fig. 12 Synthetic route to amidoflumet

Metabolism, Pharmacology and Toxicity

1. Metabolism

Metabolism of amidoflumet in rats was investigated using ^{14}C-labeled amidoflumet (^{14}C-amidoflumet).

When male and female rats were administered a single oral dose of ^{14}C-amidoflumet at 5 mg/kg or 100 mg/kg, relatively immediate absorption and metabolism of amidoflumet were observed. The excretion of the radioactivity was almost complete 7 days after the administration (total excretion rate was 96.1–100% of the administered radioactivity).

The disappearance of the administered radioactivity from the organs and tissues was relatively slower in females than in males. However, there was almost no residual radioactivity in the organs or tissues of males and females at 7 days after the administration.

The major metabolic reaction of amidoflumet was the hydrolysis of ester bond to form amidoflumet BA (benzoic acid derivative), which was further conjugated with glucuronic acid (amidoflumet BA glucuronide) and glucose (amidoflumet BA glucoside) (**Fig. 13**), then excreted into feces (bile) and urine.

Amidoflumet BA glucuronide biliary excreted into the intestine was partially-hydrolyzed to amidoflumet BA, and then excreted into feces. Some amounts of amidoflumet BA glucuronide in the intestine were reabsorbed via enterohepatic circulation.

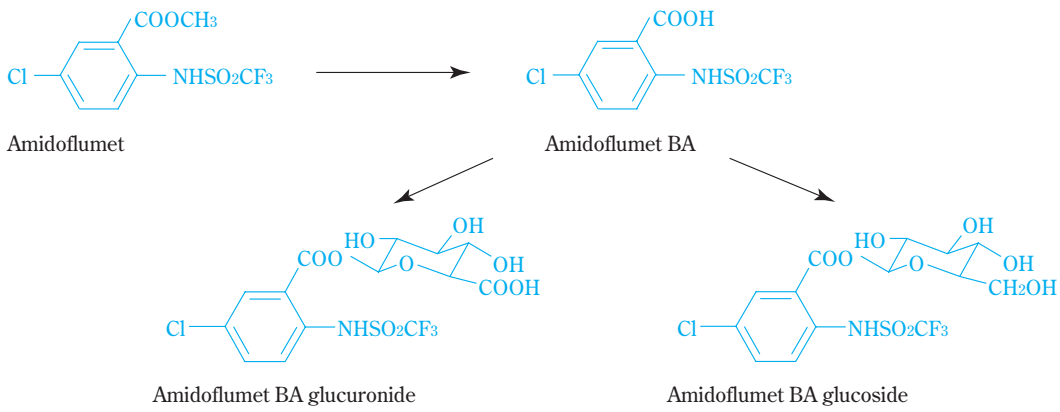


Fig. 13 Metabolic reaction of amidoflumet in rats

A sex difference was observed in the major excretory route of the administered radioactivity. The major route in males was urinary excretion (total excretion rate of radioactivity for seven days, urine: 67.0–68.8%, feces: 27.5–29.7%). In the case of females, it was fecal excretion via bile (total excretion rate of radioactivity for seven days, urine: 35.3–49.9%, feces: 43.3–59.1%). It was considered that this sex difference was due to the difference in the major elimination route used when amidoflumet BA glucuronide from hepatocytes was sorted into the systematic circulation or into the biliary excretion. In addition, the slower disappearance of radioactivity from the organs and tissues in females was due to the greater influence of enterohepatic circulation in females.

2. General Pharmacology

General pharmacological studies of amidoflumet were conducted in rats, guinea pigs, rabbits and dogs. Decreases in spontaneous movement, muscle tone and contact avoidance reactions were observed as the clinical signs and behaviors. In the central nervous system, decreased motor activity, prolongation in sleeping time and hypothermia were observed. In the autonomic nervous system and smooth muscles, inhibitory effects on histamine-, serotonin- and barium-induced contraction were observed. In the respiratory and circulatory systems, increases in respiratory rate, heart rate and blood flow, and a shortening of PR interval on the electrocardiograms were found. In metabolism of water and electrolytes, increases in urinary volume and excretion of sodium and chloride in the urine were observed. None of these changes was found at the low dosage. The changes in the *in vivo* studies were all reversible.

3. Toxicity

(1) Acute Toxicity

The approximate lethal dose was 200 mg/kg in males and 140 mg/kg in females with oral administration to rats. However, an approximate lethal dose was not able to be determined in dogs due to vomiting found in both sexes. For dermal administration to rats, the approximate lethal dose was greater than 2000 mg/kg for both sexes. For inhalation exposure, it was greater than 5440 mg/m^3 in both male and female rats (**Table 9**). The main symptoms found in rats were decreases in spontaneous activity, ataxic gait, and irregular respiration. Neural symptoms such as tremors and convulsions were observed in dogs.

Table 9 Acute toxicity of amidoflumet

Species	Administration route	Dose	Approximate lethal dose
Rat	Oral	Male : 100–750 mg/kg Female : 100–540 mg/kg	Male : 200 mg/kg Female : 140 mg/kg
Rat	Dermal	2000 mg/kg	> 2000 mg/kg
Rat	Inhalation	5440 mg/m^3	> 5440 mg/m^3
Dog	Oral	80, 400, 2000 mg/kg	Undetermined due to vomiting in both sexes

(2) Subacute and Chronic Toxicity

In the subacute and chronic toxicity studies, effects on the liver, water and electrolyte metabolism, bone marrow and red blood cells were found in animals treated with amidoflumet, and the animals made a recovery after cessation of the treatment.

Effects on the liver were found in both rats and dogs, and in rats, there were increased liver weight and hepatocyte hypertrophy. These changes were similar to the

histological changes observed for induced drug metabolizing enzyme activity after chemical treatment[5), 6)]. Metabolism studies revealed that amidoflumet is primarily metabolized in the liver ; therefore it was considered that induction of metabolic enzymes arose as an adaptive response in the body related to metabolism in the liver. Furthermore, hepatocellular vacuolation (fat vacuoles) was seen in rats, and decreases in total cholesterol, phospholipids and triglycerides were found in the blood biochemistry in both rats and dogs. Thus, it was clear that there was an indicating effect on lipid metabolism. These changes were all reversible.

In terms of the effects on water and electrolyte metabolism, changes indicating effects on body fluid balance, such as increases in water consumption and urine volume, a hypertrophy of the glomerulosa cells of the adrenal cortex, a decreased pH of the urine and decreases in chloride and potassium excretion, were found in rats. Decreased serum sodium was observed in dogs. In the general pharmacological studies, increases in urinary volume and excretion of sodium and chlorides in the urine were also observed, and this indicates that amidoflumet affects water and electrolyte metabolism. However, since there was no morphological disorder in the kidney in any of the toxicity studies, these effects were not considered to be toxicologically significant. In addition, these changes were all reversible after cessation of the treatment.

With the bone marrow, slight decreased hemopoietic cells and increased fat cells were observed in rats. These changes were possible secondary effects due to malnutrition status based on the following rationales: a) they were not always observed concurrently with alterations in circulatory blood, b) the possibility that they directly attribute to the differentiation and/or proliferation of bone marrow cells is low, and c) amidoflumet-induced inhibition of body weight gains involving decreased food consumption was observed.

Effects on the blood were observed in both rats and dogs. In rats, there were slight decreases in red blood cells, the amount of hemoglobin, mean corpuscular hemoglobin concentration and the platelet count, whereas in dogs, slight decreases in hematocrit value, the amount of hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin were observed. It was considered that there was a possibility of inhibition of the hemoglobin synthesis system of red blood cells. However, all these changes were slight and not severe to cause adverse effects in the overall body condition, and they were reversible after withdrawal of the treatment.

Besides this, hyperplasia and keratinization of epithelial cells in the larynx, cartilage necrosis, squamous metaplasia and inflammatory cell infiltration of the subepithelium were observed in the repeated inhalation toxicity study. These changes were often found when rats were exposed to irritating substances by inhalation[7), 8)], and these findings were reversible after withdrawal of the inhalation. Therefore, it was considered that these findings were local and reversible effects induced by the irritating properties.

(3) Reproductive and Developmental Toxicity

In terms of reproductive and developmental toxicity, a study of fertility and early embryonic development to implantation in rats, a study for effects on embryo-fetal development in rats and rabbits, and also a study for pre- and postnatal development including maternal function in rats were conducted (**Table 11**).

In the study of fertility and early embryonic development to implantation in rats, no test substance-related effects on fertility and early embryonic development were found.

In the study of effects on embryo-fetal development in rats, decreased fetal weight, increased skeletal variations (wavy ribs and lumbar ribs) and decreased skeletal ossification progress were found. In the study for effects on embryo-fetal development in rabbits, abortion or premature delivery related to the suppression of food consumption[9)], and increase in the rate of early resorptions were found. However, all of these changes

Table 10 Subacute and chronic toxicity of amidoflumet

Species	Administration route and duration	Dose	NOAEL
Rat	Oral (in diet), 1 month	100, 1000, 5000, 10000 ppm	Male : 1000 ppm (62 mg/kg/day) Female : 1000 ppm (66 mg/kg/day)
Rat	Inhalation, 28 days	927, 4460, 8170, 30500 μg/m^3	927 μg/m^3 (effects on larynx) 30500 μg/m^3 (effects on whole body)
Dog	Oral (capsule), 90 days	1, 3, 30 mg/kg	Male : 3 mg/kg/day Female : 1 mg/kg/day
Rat	Oral (in diet), 6 months	60, 100, 1000, 8000 ppm	Male : 1000 ppm (47 mg/kg/day) Female : 1000 ppm (59 mg/kg/day)

Table 11 Developmental and reproductive toxicity of amidoflumet

Study	Species	Administration route and duration	Dose (mg/kg/day)	NOAEL (mg/kg/day)	
Effects on fertility and early embryonic development to implantation	Rat	Oral (gavage) Male : 4 weeks before mating to termination (sacrifice) Female : 2 weeks before mating to day 7 of gestation	Male : 10, 30, 200 Female : 10, 30, 100	Parental	Systemic NOAEL Male & Female: 30 Reproductive NOAEL Male : 200 Female : 100
				Developmental	100
Effects on embryo-fetal development	Rat	Oral (gavage) Days 6-19 of gestation	4, 20, 100	Maternal	Systemic NOAEL : 4 Reproductive NOAEL : 100
				Developmental	20
	Rabbit	Oral (gavage) Days 6-28 of gestation	10, 30, 100	Maternal	Systemic NOAEL : 30 Reproductive NOAEL : 30
				Developmental	30
Effects on pre- and postnatal development, including maternal function	Rat	Oral (gavage) Day 6 of gestation to day 20 of lactation	4, 20, 100	Maternal	Systemic NOAEL : 4 Reproductive NOAEL : 100
				Developmental	20

were found in the highest dose group that showed some maternal toxicity. In addition, no teratogenicity was observed in these studies.

In the study of pre- and postnatal development including maternal function in rats, there were no effects on the maternal reproductive functions, growth, development or reproductive functions of pups although suppression of body weight gains in F1 pups was found in the dosage group that exhibited some maternal toxicity.

(4) Antigenicity

Amidoflumet did not show any potential to provoke skin sensitization (maximization test) or systemic anaphylactic reactions in guinea pigs.

(5) Irritation Effects

Amidoflumet was slightly irritating to the skin and the eyes of rabbits. Therefore, no adverse irritating effects are expected under the conditions of practical use.

(6) Genotoxicity

The results of reverse mutation tests using *Salmonella typhimurium* and *E. coli*, *in vitro* chromosomal aberration tests using Chinese hamster lung cells (CHL/IU) and micronucleus tests using mouse bone marrow were all negative (**Table 12**).

(7) Fish Toxicity

Exposure tests were conducted for 48 hours using carp (*Cyprinus carpio*koi) under static conditions in still water. The 48-hours LC_{50} value for 48 hours was determined to be 6.0 mg/l.

Table 12 Mutagenicity of amidoflumet

Study	Study design	Results
Reverse mutation (Ames test)	*S. typhimurium* : TA100, TA98, TA1535 and TA 1537 *E. coli* : WP2*uvrA* –S9 mix : 156 – 5000 μg/plate +S9 mix : 39.1 – 5000 μg/plate	Negative
In vitro chromosomal aberration	Chinese hamster lung cell (CHL/IU) Short treatment : –S9 mix : 400, 600, 800 μg/ml +S9 mix : 200, 400, 600 μg/ml Continuous treatment (–S9 mix) : 24-hour : 20, 40, 80 μg/ml 48-hour : 10, 20, 40 μg/ml	Negative
Micronucleus	Mouse (CD-1, 8-week old) 62.5, 125, 250 mg/kg (single oral administration)	Negative

Conclusion

The household mite controlling agent amidoflumet (Panduck®) developed by Sumitomo Chemical Co., Ltd., exhibits a new usefulness not seen in existing miticides. It has a greater lethal efficacy and more rapid effects for all dust mites than conventional agents. Furthermore, it has a high level of lethal efficacy for cheyletid mites that were difficult to exterminate with the agents used up to this point. Furthermore, the

stability of amidoflumet is superior and it may be adapted to various types of preparations. Since it has a high level of safety in scenarios for actual use, there are great expectations for it as an agent with particular efficacy for household dust mites. Moreover, amidoflumet is being made practical as a single active ingredient for agents that control household mites.

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