Variation in cutaneous perfusion due to synthetic pyrethroid exposure

S A FLANNIGAN AND S B TUCKER

From the University of Texas Health Science Center at Houston, Schools of Medicine and Public Health, Houston, Texas, USA

ABSTRACT Synthetic pyrethroids are neurotoxic insecticides with a low mammalian toxicity. Prior investigations have found these agents to be neither cutaneous irritants nor sensitisers. Clinically demonstrable inflammation, as judged by erythema, oedema, or vesiculation, has not been apparent. Nevertheless, paraesthesia does result from cutaneous exposure. In this investigation technical grade flucythrinate was applied to the forearms of human participants twice daily for five consecutive days. Laser Doppler velocimeter measurements were made before each topical application, with histamine phosphate being intradermally injected on termination of the study. Results of both laser Doppler velocimetry and histamine induced axon reflex vasodilatation were not statistically significant at an $\alpha = 0.05$ level. This investigation suggests that the synthetic pyrethroids have little influence on neurogenic vasodilatation on topical exposure.

Synthetic pyrethroids are neurotoxic broad spectrum insecticides. They are highly lipophilic and possess an enhanced photostability, greater insecticidal activity, and diminished mammalian toxicity. Since their initial introduction in early 1973, they have attained approximately 30% of the commercial market for insecticides by providing an excellent cost/benefit ratio for agricultural and domestic pest control.¹ Well over 1000 pyrethroids have been synthesised to date.

Synthetic pyrethroids are used for many purposes, varying from food protection to general pest control. Bernhard and Bennet found that ultra low volume applications were effective for insect control in stored products.² Recent investigations have also indicated their applicability as grain protectants.³ Furthermore, cattle ear tags are now being treated with synthetic pyrethroids as an effective restraint for horn flies.⁴ White and Benach noted that the American dog tick, *Dermacentor variabilis*, is highly susceptible to these insecticides when topically applied.⁵ Even in the domestic greenhouse these agents are effective in eradicating the white fly, *Trialeurodes vaporariorum*.⁶

The biological activity of the pyrethroid insecticides appears to be directly associated with their chemical structure.^{7,8} Almost all active pyrethroids are esters whose constituent acids and alcohols are basically inert. Electron density and polarisability are of little importance. Flucythrinate,

the synthetic pyrethroid used in this investigation, has a cyano substituent in the s-configuration of the molecular structure that tends to increase its strength by threefold to sixfold.

Common name: flucythrinate

Technical flucythrinate, $C_{26}H_{23}F_2NO_4$, has a dermal LD_{50} for the rabbit in excess of 1000 mg/kg body weight and an oral LD_{50} of approximately 75 mg/kg body weight in the rat. Cutaneous irritation and sensitisation are either negative or minimal for all pyrethroid insecticides studied.⁹ LeQuene *et al*, however, found that among 23 researchers exposed to synthetic pyrethroids, 19 experienced one or more episodes of abnormal facial sensation.¹⁰ Kolmodin-Hedman *et al* noted that forestry workers displayed symptoms that were similar to itching and burning of the skin.¹¹ Tucker and Flannigan described a variation in paraesthesia among agricultural applicators of fenvalerate.¹² The susceptibility of the workers did not seem to be based on race, personal history of atopy, or the number of exposures.

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Few investigations have attempted to correlate neurogenic vasodilatation with topical exposure to the synthetic pyrethroids. Neurogenic vasodilatation, or flare, is a component of the inflammatory response to tissue injury. All prior dermal testing with the pyrethroids has been in the animal model, neglecting the fact that reactions to vasoactive compounds differ remarkably from species to species.¹³ Furthermore, flare reactions are often quite difficult to observe in the animal model. Therefore, based on the low inherent mammalian toxicity of the synthetic pyrethroid insecticides, repeated daily applications of flucythrinate were made to human participants to see if repeated dermal exposure would result in neurogenic vasodilatation.

Material and methods

Four healthy volunteers, aged from 22 to 38, took part in this study. Technical grade flucythrinate was diluted in absolute ethanol, yielding a concentration of 13.84 mg/cm² on application. The placebo was absolute ethanol. This research was reviewed and approved by the Committee for the Protection of Human Subjects, Health Science Center at Houston.

Each participant received a topical application of either diluted technical grade flucythrinate or absolute ethanol to an area of 2.85 cm² on the anterior surface of a forearm. On the opposite forearm, the corresponding preparation was applied. All applications were double blind and repeated twice daily, at 0800 and 1600, for five consecutive days.

Before the application, the forearms of each participant were first dampened with water and then dried. A disposable tuberculin syringe delivered 0.05 cc of either the test material or placebo to a designated site which was then semi-occluded with gauze and Scanpor tape. Participants were instructed to wash both forearms with soap and water about one hour before evaluation and reapplication. All measurements were made in a single well ventilated room under reasonably constant temperature and humidity, to which participants were acclimatised for 15 minutes before testing. Skin blood flow was measured in four different positions on each test site using a laser Doppler velocimeter (LDV). Before any blood flow measurements, the capillary perfusion monitor was zeroed using its internal circuitry. The values displayed by the system were the root mean squares of the photodiode signals and were normalised to the intensity of the backscattered skin surface illumination. The maximum depth of penetration was estimated at 1.5 mm. All voltage output was collected by an IBM personal computer that was integrated with the LDV through a single board analogue and digital I/O system.

At the end of the fifth day, both forearms were graded with the LDV and then injected intradermally with 0.02 ml of 1:10 000 histamine phosphate directly into the centre of each site. Weal and flare reactions were outlined at three minutes, traced on to transparent plastic film, and measured on a digitiser.

The double blind code was broken at the end of the five days. Statistical analyses used an analysis of variance (ANOVA) with repeated measures for the evaluation of the data acquired through laser Doppler velocimetry, as well as the weal and flare reactions at three minutes. Differences at individual time points between treatment means were determined in all circumstances through the application of a protected least significant difference (PSD) multiple comparison test.

Results

Ten topical applications were made to each participant in this investigation. Blood flow, measured by LDV, appeared to vary little between treated and control sites. A statistical significance ($p \leq 0.05$) between sites was not apparent for any of the days of the investigation nor over the cumulative period. Some fluctuation between morning and afternoon values occurred for both regimens (figure).

Paraesthesia was noted by each participant on the treated site for the duration of the investigation. Cutaneous sensations began about four to five hours

Variation in cutaneous perfusion from repeated application of technical flucythrinate or placebo.

after the initial application and were described as a burning to tingling sensation that eventually degraded to a mild degree of pruritus by day 3. Clinically demonstrable inflammation, as judged by erythema, oedema, or vesiculation, was not apparent.

Intradermally injected histamine evoked flare, weal, and itching in both normal and treated skin of all participants. Statistical analyses of the weal and flare at three minutes after injection was not significant (table).

Analyses of variance for flare and weal from intradermally injected histamine phosphate

Reaction	Cell means	
	Technical flucythrinate (13.84 mg/cm ²)	Placebo (absolute ethanol)
Flare (cm ²)	17.48	17.61
	+-----+-----+	
Weal (cm ²)	1.09	0.99
	+-----+-----+	

+----+: Not statistically significant at $p \leq 0.05$.

Discussion

Our current knowledge concerning the site and mechanism of the synthetic pyrethroid insecticides indicates that the resultant dysaesthesia is due to a specific interaction between the pyrethroid molecules and sodium channels of the nerve axon. Based on the universal nature of nerve irritability and conductivity, the action of these insecticides should not be considered restricted to any particular species or region of the nervous system. On the basis of prior voltage clamp studies, treatment with pyrethroids has been shown to result in the sodium channels closing considerably more slowly than usual.¹⁴ As a result, a gradually decomposing inward sodium current remains after termination of the depolarisation phase. Various rates of decay exist for the sodium tail current between the various pyrethroids.

In this investigation cutaneous perfusion was evaluated by LDV after topical application of technical grade flucythrinate. The laser source was a 5 mV He/Ne laser conveyed to the skin surface through a quartz optical fibre and backscattered from both the fixed cutaneous surface and flowing erythrocytes. A second internal fibre in the probe head gathered the reflected light from both surfaces and returned it to a photodiode for mixing and interfacing. The actual output from the system was the root mean square of the photodiode signal. The values were expressed in millivolts and directly proportional to the flow velocity.¹⁵ The maximum depth of penetration was 1.5 mm.¹⁶

When human skin is exposed to what is considered to be a chemical irritant, the site and surrounding region for several centimetres often becomes erythematous. This flare, which is a component of the inflammatory response, represents neurogenic vasodilatation.¹⁷ Fearn *et al* found that the topical application of xylene produces, in addition to this inflammatory response, an immediate and pronounced rise in blood pressure providing further evidence of the involvement of the nervous system.¹⁸ In this investigation flucythrinate was topically applied daily and its effect on cutaneous perfusion monitored. The blood supply remained consistent throughout the investigation when comparison was made between control and test sites over all participants.

The peripheral sensory nervous system has been shown to play a key part in the inflammatory process. It functions not only as a receptor site for pain but also as control for vascular permeability. Several experimental studies have shown that inflammatory responses to certain chemical irritants can be affected, or even prevented, by prior treatment with capsaicin, which presumably desensitises the afferent nerve endings.^{19,20} In view of this, a question arose as to whether the axon reflex flare in human skin could be diminished by pretreatment with a synthetic pyrethroid. Even though topically applied flucythrinate did result in a burning to tingling sensation throughout the course of the investigation, it did not inhibit the histamine induced axon reflex vasodilatation.

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