# *trans*-1,4,5,6-Tetrahydro-2-(3-hydroxystyryl)-1-methyl pyrimidine (CP-14,445), A New Antiwhipworm Agent (36151)

H. L. Howes, Jr.
(Introduced by J. E. Lynch)
*Pfizer Inc., Groton, Connecticut 06340*

With the exception of *Trichuris* sp. and *Strongyloides* sp. helminths which reside in the alimentary canal of their hosts have proven to be fairly vulnerable to a variety of anthelmintics. Pyrantel and its *ortho*-methyl homolog morantel are potent examples of agents which effectively control nematodes, such as strongyles in the small intestines of sheep (1), swine (2), and dogs (3) and pin worms in the cecum and lower bowel of man (4) and mice (3), but are not effective against whipworms. The close association to host tissue typical of whipworms and other trichurids renders them difficult to treat and, Standen theorizes (5), this is one reason for the shortage of effective agents. Another reason has been the lack of a reliable small animal test, the existence of which would permit many compounds to be readily evaluated. For even though *Trichuris muris* can be maintained in mice in the laboratory, it has been thought to have low predictive value as an anthelmintic test model (5).

CP-14,445, the *meta*-oxyphenyl analog of pyrantel, has been found to be a very potent antiwhipworm agent, and its activity in mice and dogs is the subject of the present report.

*Materials and Methods.* Trichuris muris *in mice.* Whipworm infections were established using procedures previously described (6, 7). Briefly, pinworm free, CF No. 1, male mice were inoculated orally with 150–300 embryonated ova. A 230 mg/kg dose of cortisone acetate was administered subcutaneously 2 and 10 days postinfection (pi) to prevent the spontaneous elimination of worms which normally occurs 2 to 4 weeks pi (8). Test compounds were administered either in mouse meal (Purina) or by gavage in 1% methylcellulose. Chemotherapeutic activity was expressed as a percentage reduction in the mean number of worms (worm burden) in a treated group relative to that of an untreated contol group 30–35 days pi. When it was considered useful, standard deviations (SD) and $p$ values (Students' $t$ test) were calculated.

*Other helminth infections in mice. Nematospiroides dubius* and *Hymenolepis nana* infections were artificially induced in CF No. 1, male mice with naturally occurring *Syphacia obvelata* infections, thus producing a triple infection. *Trichinella spiralis* infections were also established in CF No. 1, male mice by inoculating them orally with larvae obtained from donor mice. The effectiveness of compounds administered by gavage in 1% methylcellulose was measured on the basis of reduction in worm burdens posttreatment (post-Rx). Details on the methods of infection, treatment, and drug evaluation are published elsewhere (3, 9).

*Intestinal helminth in dogs.* Naturally infected mongrel dogs were grouped according to their worm burdens as determined by pre-Rx ova counts (10). After fasting overnight, drug was administered orally in gelatin capsules and the dogs were observed periodically for the next several hours for signs of toxicity. Activity was measured on the basis of 72 hr post-Rx ova counts, worms passed in feces during the 72 hr post-Rx period, and worms present at necropsy.

*Results.* On the basis of $ED_{90}$ values (mg/kg of base) (Table I) CP-14,445·HCl and pyrantel HCl were equipotent against the enteral stages of *T. spiralis*. However, their patterns of activity and potencies against the three species of worms in the triple infection were very dissimilar. Pyrantel HCl was much more potent (17 times) against *N. dubius* than it was against adult

TABLE I. Therapeutic Activity of Pyrantel HCl, CP-14,445 • HCl, and CP-14,445 • Pamoate Against Various Helminths [a] in Mice.

| Helminth | Oral $ED_{90}$ (mg/kg) of base material | | |
|---|---|---|---|
| | Pyrantel HCl | CP-14,445 • HCl | CP-14,445 • Pamoate |
| Triple infection: | | | |
| *H. nana* | > 85.5 | 52.7 | > 500 |
| *N. dubius* | 5.1 | 105.4 | > 500 |
| *S. obvelata* adults | 85.5 | 26.5 | > 62.5 |
| immature | > 85.5 | 105.5 | > 500 |
| *T. spiralis* 2 hr pi | 41.4 | 41.4 | NT |
| 144 hr pi | 82.8 | 82.8 | NT[b] |

[a] Mean worm burdens: *H. nana*, 13.4; *N. dubius*, 19.5; *S. obvelata*, 21.4 (adults), 31 (immature); *T. spiralis*, 208; [b] Not tested.

*S. obvelata*. Whereas CP-14,445, whether soluble (HCl) or relatively insoluble (pamoate), was more potent (4 and 8 times, respectively) against adult *S. obvelata* than it was against *N. dubius*. Furthermore, CP-14,445·HCl had an $ED_{90}$ of 52.7 mg/kg of base against *H. nana*, but pyrantel HCl was inactive at its maximum tolerated dose (85.5 mg/kg of base). Thus, even though CP-14,445 was not a particularly potent analog of pyrantel, the fact that there was a reversal in the order of sensitivity of *S. obvelata* and *N. dubius* and activity against *H. nana* prompted further study.

The antiwhipworm activity of single oral doses of various anthelmintics was measured in the *T. muris* mouse model (Table II). Only methyridine, CP-14,445·HCl, and CP-14,445·pamoate reduced worm burdens by 90% or more, with CP-14,445 doing so at doses below 2 mg/kg. Multiple doses of phthalofyne and dithiazanine iodide are known to be effective against other *Trichuris* species (11, 12) and a low, but significant ($p = .05$) order of activity against *T. muris* was detected with only single doses. Similar results were obtained with relatively high doses of pyrantel HCl and thiabendazole, reflecting the antitrichurid activity also seen against *T. spiralis*. Resin formulations of dichlorovos (Shell's Atgard or Task) are active against whipworms in many domestic animals (13) and, even though in order to dose mice it had to be used in its unformulated (liquid) state, it too was active against *T. muris*.

Activity against the earliest stages of *T. muris* was measured in feed incorporation studies (Table III). All 3 tetrahydropyrimidines (CP-14,445, pyrantel, morantel) and thiabendazole completely prevented the establishment of mature worms. However, the superior potency of CP-14,445·HCl observed against mature *T. muris* was also evident against the earliest stages (0–7 days pi).

CP-14,445 activity against *T. muris* was confirmed in studies against *T. vulpis* in dogs (Table IV). A single dose of 12.5 mg/kg of CP-14,445·HCl (10.6 mg/kg of base) and 50 mg/kg of CP-14,445·pamoate (26 mg/kg of base) completely eliminated the whipworm burdens of naturally infected dogs. At the emetic dose of 50 mg/kg of CP-14,445·HCl a few tapeworms, hookworms, and ascarids were passed but not at the 12.5 mg/kg dose. Even a single dose of 2.0 mg/kg of the HCl and 10 mg/kg of the pamoate caused significant numbers of whipworms to be eliminated. Virtually no whipworms were passed by unmedicated dogs.

When a 3 mg/kg dose of CP-14,445·HCl was combined in a single gelatin capsule with 2 mg/kg of morantel HCl, the combination affected a > 95% reduction in hookworm, ascarid, and whipworm ova counts and caused almost all the worms to be passed during the 3 day post-Rx period (Table IV). These data compare very favorably with those obtained with formulated dichlorovos (Task) at 40 mg/kg.

*Discussion*. The results show that CP-14,445 is a uniquely potent antiwhipworm compound which requires neither elaborate for-

TABLE II. Therapeutic Activity of CP-14,445 and Selected Anthelmintics in Mice (3–8/group) Infected with *T. muris*.

| | Expt. Compound | Single dose (mg/kg, po) | Total mice | Mean worm burden | SD [a] | *p* value [b] | Percentage reduction |
|---|---|---|---|---|---|---|---|
| I | Dithiazanine iodide | 10 | 5 | 81 | 49 | .05 | 36 |
| | Phthalofyne | 10 | 6 | 81 | 36 | .05 | 36 |
| | l-Tetramisole | 20 | 6 | 107 | 28 | >.10 | 15.1 |
| | Thiabendazole | 400 | 6 | 73 | 78 | .01 | 42.1 |
| | Unmedicated | — | 6 | 126 | 13 | — | — |
| II | Pyrantel HCl | 85.5 | 3 | 38 | 11 | .005 | 54.2 |
| | Unmedicated | — | 5 | 70.4 | 13 | — | — |
| III | Dichlorovos | 50 | 2 [c] | 47 | 3.6 | .005 | 56.1 |
| | | 12.5 | 6 | 93 | 16 | .10 | 13.1 |
| | | 3.12 | 6 | 111 | 21 | — | 0 |
| | Unmedicated | — | 8 | 107 | 14 | — | |
| IV | Methyridine | 800 | 8 | 0.25 | — | — | 99.5 |
| | | 200 | 8 | 6.5 | — | — | 86.3 |
| | Unmedicated | — | 10 | 47.4 | — | — | — |
| V, VI, and VII | CP-14,445 · HCl | 8.5 | 8 | 0 | — | — | 100 |
| | | 3.4 | 16 | 2.3 | — | — | 94.9 |
| | | 1.7 | 24 | 3.0 | — | — | 93.3 |
| | | 0.85 | 8 | 22.3 | — | — | 55 |
| | CP-14,445 · pamoate | 10.3 | 8 | 0.2 | — | — | 99.7 |
| | | 6.4 | 6 | 0 | — | — | 100 |
| | | 2.1 | 14 | 13.2 | — | — | 71.2 |
| | Unmedicated | — | 24 | 47.8 | — | — | — |

[a] Standard deviation.
[b] Students' *t* test.
[c] Lethal of 4 to 6 mice treated.

mulation nor multiple dosing to be effective. Further, they show that, just as the discovery of pyrantel larvacidal activity against *Ascaris suum* contributed significantly to the validation of the predictive value of the *A. suum*-mouse test model (14), studies with CP-14,445 have lent validity to the *T. muris*-mouse model. Keeling (15) was compelled to use mice with relatively uneven worm burdens and multiple and/or large doses of compounds with limited antiwhipworm activity. As a result he concluded "attempts to assess the validity of *T. muris* have produced some interesting but inconclusive results." However, with the availability of procedures which produce more consistent worm burdens (6–8), even compounds less effective than CP-14,445 can be evaluated.

Since pyrantel, morantel, and thiabendazole are known to be especially effective against the earlier, enteral stages of *T. spiralis* their activity against the earlier stages of the trichurid, *T. muris*, was not surprising. The superior potency of CP-14,445 was also not surprising. However, all previous anthelmintic studies in mice, dogs, sheep, and swine against various nematodes have shown pyrantel to be less potent than morantel (1, 16). Thus, pyrantel superiority over morantel against the earliest stages of *T. muris* was unexpected.

The confirmation of CP-14,445 antiwhip-

TABLE III. Prophylactic Activity of CP-14,445 and Selected Anthelmintics in Feed (—1 to 7 days pi) Against *T. muris* [a] in Mice (6-9/group).

| Compound | In feed (%) | No. of trials | Total mice | Percentage reduction |
|---|---|---|---|---|
| CP-14,445 • HCl | 0.05 | 3 | 16 | 100 |
| | 0.005 | 2 | 14 | 97.5 |
| | 0.002 | 1 | 8 | 78.1 |
| | 0.0012 | 1 | 7 | 59 |
| Pyrantel HCl | 0.05 | 5 | 30 | 100 |
| | 0.025 | 1 | 6 | 99 |
| | 0.01 | 1 | 6 | 80.1 |
| | 0.0025 | 1 | 6 | 32.6 |
| Morantel HCl | 0.05 | 5 | 30 | 91.1 |
| | 0.025 | 1 | 6 | 43 |
| | 0.01 | 1 | 6 | 41.9 |
| | 0.0025 | 1 | 6 | 44 |
| Thiabendazole | 0.05 | 1 | 8 | 90.4 |
| | 0.0125 | 1 | 7 | 14 |

[a] Unmedicated control mice mean worm burden (5 trials): 76.7.

worm activity in mice by studies in dogs is encouraging, in that most of the currently available antiwhipworm agents are also effective in dogs. The fact that CP-14,445 loses potency as pamoate suggests the possibility that it may exert its effect systemically thus favoring the more readily absorbed HCl salt. However, both forms, CP-14,445·HCl and CP-14,445·pamoate, are markedly more potent than dichlorovos (Task). Whether or not its superiority is retained in other animals remains to be demonstrated, but results from

TABLE IV. Therapeutic Activity of CP-14,445 Against Helminths in Naturally Infected Dogs.

| | | | | Ova | | Worms Post-Rx | |
|---|---|---|---|---|---|---|---|
| Compound | (mg/kg) | Helminth | No. of dogs | Pre-Rx (ova/g) | Post-Rx (% red) | No. passed | No. at necropsy |
| CP-14,445 • HCl | 25 | | 6 | 1197 | 95 | 22 | 0.7 |
| | 12.5 | | 6 | 586 | 95 | 34 | 0.0 |
| | 3.12 | | 6 | 450 | 87 | 17 | 19 |
| | 2.0 | Whipworm | 8 | 368 | 75 | 8.7 | 21 |
| CP-14,445 • pamoate | 50 | | 5 | 468 | 91 | 41.8 | 0 |
| | 20 | | 6 | 1236 | 80.5 | 146 | 19 |
| | 10 | | 5 | 1286 | 58 | 14.2 | 44.2 |
| Unmedicated | — | | 20 | 1334 | — | 0.10 | 58 |
| CP-14,445 • HCl | 3 | Hookworm | 5 | 2545 | 97.4 | 4.2 | 0.6 |
| | + | Ascarids | 2 | 342 | 100 | 7.0 | 0.0 |
| + morantel HCl | 2 | Whipworm | 6 | 471 | 95.5 | 24.0 | 7.7 |
| Dichlorovos | 40 | Hookworm | 2 | 943 | 100 | 11.0 | 0.0 |
| (Task) | | Ascarids | 2 | 1063 | 81 | 40.0 | 5.0 |
| | | Whipworm | 2 | 258 | 50 | 31.5 | 10.0 |

preliminary studies in monkeys (Howes, unpublished) and swine (Conway, unpublished) show CP-14,445·HCl to be at least as potent as it is in dogs. Finally, the fact that a combination of CP-14,445·HCl and morantel HCl has the same spectrum and 8 times the potency of dichlorovos (Task), broadens the scope of uses to which CP-14,445 might be put.

*Summary.* The antiwhipworm activity of CP-14,445, the *meta*-oxyphenyl analog of pyrantel, was described. In dogs it was more potent as the HCl salt than as the pamoate, but both forms were more potent than dichlorovos (Task). Dosage response data obtained from mouse and dog studies indicate that the *T. muris*-mouse infection, as a test model for antiwhipworm studies, has predictive value.

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