PP796 – toxicology summary.

1. History

PP796 (or ICI 63,197) is a triazolo-pyridine. The compound was originally discovered by ICI Pharmaceuticals and was extensively studied as a potential drug for the relief of asthma. Mammalian toxicology studies were completed to the satisfaction of the UK Committee for the Safety of Medicines, which granted a Clinical Trials Certificate, enabling clinical trails to take place. During the clinical trials, it became apparent that PP796 had an unexpectedly high emetic effect in humans. It was therefore withdrawn from further development as a drug. Its emetic properties did however indicate considerable potential for use with a pesticide formulation.

2. General Mode of Action.

PP976 is a powerful emetic by all routes of administration. Emesis occurs as a reflex response to a rapid increase in cyclic AMP in the blood, caused by inhibition of the enzyme phosphodiesterase.

3. Pharmacology and Biochemistry Reported in RIC4223 – FG Farrell - 1970

ICI 63,197 is well absorbed following oral administration in the mouse, rat, guinea pig, dog and rhesus monkey. With the exception of the rat, at least 70% of the administered dose was passed in the urine by 48h. (The rat differs from the other species in passing a large proportion (43%) of the oral dose in the faeces.) It has been shown that biliary excretion is the major route in the rat and whole body autoradiography indicates that biliary excretion and reabsorption occurs in mice.

ICI 63,197 is extensively metabolised in all the above species, with the urine containing ICI 68,916, a metabolite in which the methyl group of ICI 63,197 has been hydroxylated. In guinea pigs, it has been shown that serum and tissue levels of total radioactivity are steady over the period 0.25 – 4h after oral administration, with maximum levels at about 1h. The maximum serum level of ICI 63,197 is higher in guinea pigs (0.87 μg/ml) than in rats (0.17 μg/ml) or mice (0.06 μg/ml) after an oral dose of 1 mg/kg. ICI 68,916 is a minor component in the serum of all three species – with 5, 4 and 7% of the total radioactivity in serum in the guinea pig, rat and mouse respectively.

Measurement of the concentration of ICI 63,197 in the serum of rats and dogs after prolonged dosing showed:

- No difference in the levels between the sexes
- A linear dose – peak serum level response and a linear dose – area under the curve response in dogs throughout the range of doses tested (i.e. 0.15-1.5 mg/kg/day) with slopes of 0.26 μg/ml per 1 mg/kg dose and 1.18 μg.hr/ml per 1 mg/kg dose respectively. Similar effects were noted in rats in the dose range up to 1.25 mg/kg with slopes of 0.11 μg/ml and 0.52 μg.hr/ml per 1 mg/kg dose i.e. about half the response seen in the dog.
- A biological half-life of <3h in the dog.

There was no evidence to suggest that serum concentration significantly increased or decreased after prolonged administration, hence PP796 is unlikely to be cumulative.

4. Acute toxicity data

Acute oral toxicity to the rat – CTL/L/2034 – VM Davidson 1988
Groups of 5 male and 5 female rats received single oral doses of 100, 150 and 200 mg/kg/body weight of PP796. Moderate signs of toxicity were seen at 100 mg/kg, but all animals had recovered by day 7. Marked signs of toxicity were seen at both 150 and 200 mg/kg, with 9/10 animals dosed with 150 mg/kg, and 8/10 animals dosed with 200 mg/kg, being found dead or killed in extremis day 2. (All surviving animals had recovered by day 10.) Clinical sign of toxicity included: decreased activity, salivation, upward curvature of the spine, increased breathing rate, ptosis and stains around the mouth and nose. With no significant findings at post mortem, the median lethal dose is estimated as being between 100 and 150 mg/kg/bodyweight.

Acute dermal toxicity to the rat – CTL/L/2004 – VM Davidson 1988
2000 mg/kg/bodyweight of PP796 was applied to the skin of 5 male and 5 female rats for 24h, washed off, and the animals observed for signs of toxicity for 14 days. Other than an observation of slight erythema seen in one male rat day 2, no signs of dermal irritation were noted. There were no mortalities, and with no macroscopic effects at post mortem, the acute dermal median lethal dose is considered to be > 2000 mg/kg/day.

Skin Irritation -CTL/T/1277 - SE Moses 1979
ICI 63,197 caused slight irritation to rat skin and some evidence of dermal toxicity following repeated occluded application. Signs of irritation were evident after the 4th application when all animals developed erythema. In addition, all animals looked thin after the 5th application, one was subdued and another was hunched. One animal was found dead on the last day of study (after a total dose of 0.6 mg/kg.) Histopathological examination of the skin and selected major organs confirmed the irritant effect. With no obvious signs of chemical toxicity, the only systemic effects were severe involution of the thymus and spleen.

Eye Irritation - CTL/T/1277 - SE Moses 1979
Instillation of ICI 63,197 into the eyes of rabbits caused moderate initial pain and slight irritation. Treated eyes were examined at 1-2h, and at 1, 2, 3, 4 and 7 days post instillation. Although no corneal damage was noted, transient iridial and conjunctival reactions were observed. With all signs of irritation clearing by day 2, ICI 63,197 is considered a slight eye irritant.

Skin Sensitisation Potential - CTL/T/1277 - SE Moses 1979
ICI 63,197 has tested negative in a Stevens Ear/Flank test in guinea pigs and as such is not considered to be a strong skin sensitiser.

5. Short-term predictive tests for carcinogenicity

Results from the Ames test - CTL/P/338 – E Longstaff 1977

PP796 is non-mutagenic. PP796 has tested negative in Salmonella Ames tests, both in the presence and absence of metabolic activation (Arochlor induced liver S9 fraction) with each of 5 tester strains (TA98, TA100, TA1535, TA1537 and TA1538).

6. Sub chronic toxicity data

Rat – TPR/194

10 male and 10 female rats were orally dosed with 0, 0.25, or 1.25 mg/kg ICI 63,197 daily for 3 months. 15 male and 15 female rats were similarly exposed to 5 mg/kg. At the end of the 3-month dosing period, 5 male and 5 female rats previously exposed to 5 mg/kg ICI 63,197 were maintained without treatment for a further 12 weeks to assess reversibility.

There was a slight reduction in body weight gain in top dose male rats. Many top dose and a few mid dose rats salivated after dosing the first few weeks of treatment, but thereafter salivated before dosing. There were no treatment related effects on haematology (haemoglobin, packed cell volume, total white cell count, differential white cell count, platelets and mean cell haemoglobin) or on urine analysis. In terms of clinical chemistry, no treatment related effects were observed in AST, ICD or total protein. Slightly elevated levels of alkaline phosphatase were seen in male and female rats treated with 5 mg/kg ICI 63,197 on day 22. By day 36, the levels were statistically significantly different from controls (Males $P<0.05$; females $P<0.001$), but by day 85, had returned to normal. Significantly increased serum urea levels were noted in female rats exposed to 5 mg/kg ICI 63,197 day 36 ($P<0.001$) and day 85 ($P<0.01$). Slightly increased serum urea levels were noted in male rats day 36 only ($P<0.05$).

At study termination (and termination of the recovery animals) there were no effects on organ weights and no histological changes attributable to treatment.

Dog – TPD/64

4 male and 4 female beagles were orally dosed with capsules containing 0, 0.15, 0.5 or 1.5 mg/kg ICI 63,197 daily for 3 months. (1 male and 1 female top dose animals were maintained on study for a further 6 weeks after dosing to assess recovery.)

After the 5th week of treatment, many top dose animals salivated profusely before dosing. (One male from the same group refused to eat days 9 and 10 of treatment.) Vomiting occurred sporadically in 6 top dose and 3 mid-dose dogs from day 9 onwards. (One female top dose dog that was sick on several occasions and passed blood in its faeces was found to have an ileo-caecal intussusception at post-mortem – a relatively common abnormality in this strain of dog. Examination of this animal's bone marrow smear showed a megaloblastic hyperplasia – a finding consistent with poor intestinal absorption due to the ileo-caecal ulceration.) Weight gains were similar in both control and treated males, while top dose females lost weight sporadically. There were no treatment related effects on haematology, urine analysis, clinical chemistry or clinical pharmacology. Analysis of serum level concentrations showed ICI 63,197 to be well absorbed via the oral route.

At study termination (and termination of the recovery animals) there were no effects on organ weights. Macroscopically, many of the animals (both control and treated) were observed to have

reddish areas in the lungs. These patches of pneumonia or nodules of inflammatory cells were attributed to the presence of nematodes caused by the animals not having been treated with anti-helminthics prior to the start of dosing. One additional top-dose female had a small cystadenoma in the thyroid.

Other than a similar nematode-related bronchopneumonia, no pathological changes attributable to ICI 63,197 were noted in the recovery animals.

In conclusion then, ICI 63,197, when administered to rats, mice and dogs at several times the therapeutic level produced no pathological changes, which could be attributed to treatment; the only effects being vomiting in dogs, and elevated serum urea levels in female rats.

7. Developmental Toxicity studies

Rabbits and rats have been used to evaluate the effects of ICI 63,197 on pregnant animals during organogenesis. No deformities were observed in either rat or rabbit offspring, but at high doses in the rabbit, ICI 63,197 was toxic to the dam resulting in spontaneous abortions.

Rabbits - RIC4224 - FG Farrell – 1970
Groups of 12 female rabbits were orally dosed days 6-18 of pregnancy with 0, 0.25, 0.75 and 1.25 mg/kg ICI 63, 97. Half the animals in each group were killed day 28 and the foetuses removed. The dams were examined for signs of drug toxicity and macroscopic abnormalities. The foetuses were examined for soft tissue abnormalities before subsequent processing for skeletal examination. The remaining rabbits were allowed to litter and rear their offspring to 4 weeks post partum.
Dosing with 1.25 and 0.75 mg/kg caused an increase in the number of reabsorptions. (No reabsorptions were seen in at the 0.25 mg/kg level.) Two rabbits receiving 1.25 mg/kg aborted day 20, and another one when killed day 29, had 6 reabsorptions and no viable foetuses. Of those receiving 0.75 mg/kg one died day 18 (having 8 foetuses in utero) and another littered, but ate all the offspring. The two higher dose levels also produced anorexia. Fewer offspring survived to 28 days of rabbits treated with 0.75 mg/kg. Only a small number of deformities were detected, including the presence of extra ribs, a common finding in this stain of rabbit.

ICI 63,197 induces vomiting in dogs at high doses. Although rabbits cannot vomit, the high doses in this study resulted in poor appetite/anorexia.

In conclusion, ICI 63,197 is not teratogenic to rabbits, producing maternal toxicity at 1.25 and 0.75 mg/kg and only minimal foetal toxicity. (NOEL = 0.25 mg/kg/day)

Rats - FG Farrell – 1970
Groups of 20 female rats were orally dosed days 6-15 of pregnancy with 0, 0.25 and 1.25 mg/kg ICI 63,197. Half the rats were killed one day prior to parturition and the foetuses examined for soft tissue changes before being processed for skeletal examination. The remaining rats were allowed to litter and rear their offspring to weaning.

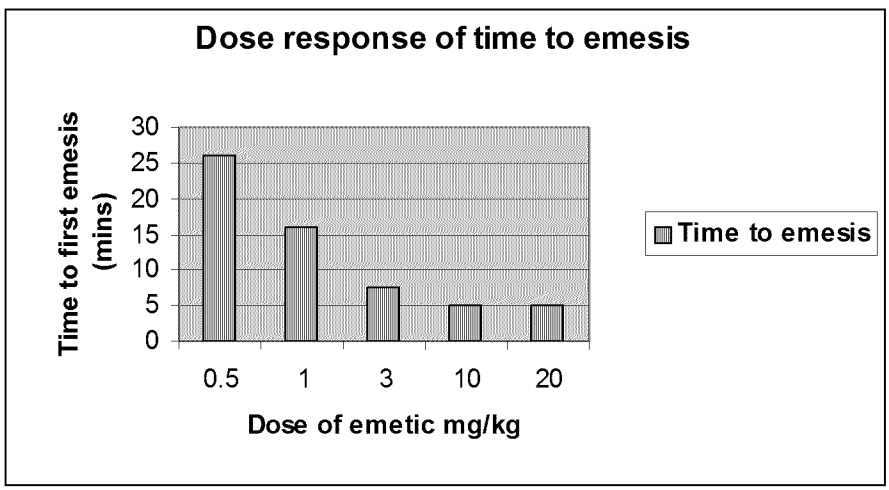
ICI 63, 197 had no significant effect on stillbirths, reabsorption rates, litter size or mean offspring weight. There was however evidence of anorexia and a reduction in bodyweight gain in top dose females. Skeletal and soft tissue changes were within normal limits for the strain of rat. In conclusion, ICI 63,197 was not teratogenic to the rat and had little effect on pregnancy, littering or weaning. NOEL = 0.25 mg/kg/day)

8. Carcinogenicity

Carcinogenicity study in mice - In RIC4222- BJ Leonard 1977
25 male and 25 female mice per group, plus controls were exposed to 5 and 20 ppm (1.25 and 5 mg/kg/day) ICI 63,197 in the diet for approximately 78 weeks. Although survival was good, statistically significant dose related reductions in bodyweight were evident at the highest dose level. With no significant difference in the tumour incidence between control and treated animals, it may be concluded that ICI 63,197 is not carcinogenic to mice. (NOEL = 1.25 mg/kg/day)

9. Effectiveness as an emetic

PP796: Emetic dose response study in dogs - CTL/T/2459 –A Brammer, M Robinson 1986
The emetic response of dogs to a range of single oral dose levels of PP796 (0, 0.1, 0.5, 1.0, 3.0, 10.0 or 20.0 mg/kg) has been investigated. Emesis and subdued behaviour was evident at all except the lowest dose level; the onset of vomiting, duration and severity being dose related. Diarrhoea and defaecation of mucus was evident in some dogs at 1.0 mg/kg, with severe vomiting at dose levels of 3 mg/kg and above. By 6 h all dogs had recovered. With no other treatment related effects, 0.5 mg/kg is considered to represent the minimal effective dose and 20 mg/kg the maximum tolerated dose level (based on the severity of the effects). The time interval between administration of PP796 and vomiting was reduced to within 10 mins by increasing the dose to 3 mg/kg, and to within 5 mins following doses of 10 or 20 mg/kg. (This reduction in the time taken being associated with an increase in the severity of the clinical effects at 10 and 20 mg/kg.)



PP796: Effect of stomach status on emetic efficacy in dogs - CTL/T/2452 –A Brammer, M Robinson 1986

The aim of the experiment was to investigate the effect stomach status has on PP796 emesis. PP796 was administered by oral gavage to dogs, at a known emetic dose of 3 mg/kg following pre-treatments of feeding food (to give a full stomach), administering COMPLAN solution (to give a liquid bolus) and withdrawal of food for 24h (to give an empty stomach). 3 mg/kg caused emesis regardless of stomach state though emesis was greatest when dosed on an empty stomach. The presence of food in the stomach enabled the dog to vomit productively thus avoiding the episodes of prolonged and marked retching seen when PP796 was given on an empty stomach.

IV administration of ICI 63,197 to beagle dogs - PH23517 - DE Case, D Dunlop 1977

In species, which vomit (e.g. pig, dog, monkey and man), a rise in plasma levels is associated with the onset of vomiting. Beagle dogs were exposed to 4.25, 4.57 and 5.76 mg/kg ICI 63,197 via an IV infusion for ½, 1 or 2h respectively. (The aim being to achieve plasma levels of 0.4 μg/ml – a level known to produce vomiting in dogs following an oral dose.) Vomiting generally occurred within 15 mins of dosing and was repeated 4 or 5 times within the first hour. The severity of the clinical effects (agitation, restlessness, panting, increased heart rate, salivation, vomiting and defaecation) being related to blood levels. Thereafter the effect ceased, probably as a consequence of the rapid metabolism and excretion of the compound. Evidence for the action of PP796 being centrally mediated is provided by the rapid onset of vomiting, the absence of irritant effects and the production of vomiting in dogs following IV administration at plasma levels similar to those producing the effect after oral administration.

The toxicity of orally administered emetic PP796 in cynomologus monkeys - CTL/C/613 – DA Purser et al 1978

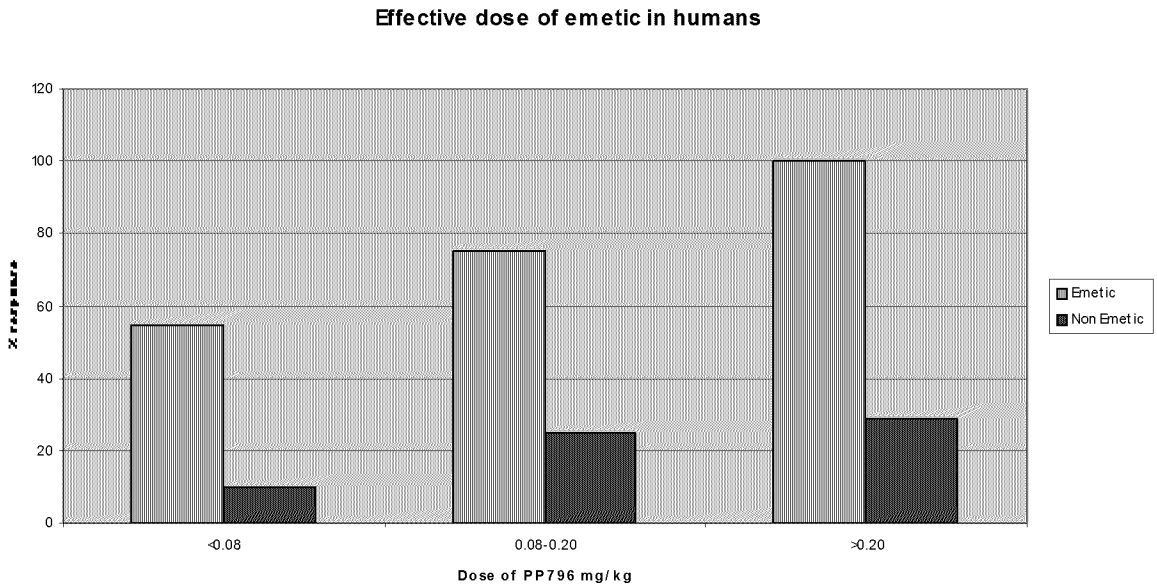
10 cynomologus monkeys (Macaca fascicularis) received a single oral dose of 100mg/kg bodyweight PP796. 9/10 animals vomited 4-43 minutes after dosing, with most animals vomiting 2-3 times. The remaining animal failed to vomit. 4/10 animals (including the one that didn't vomit) died within 24h of dosing. Surviving animals began to recover between 4 and 6 h after dosing and appeared to be completed recovered by 24h.

The concentration of PP796 required to produce emesis in man - CTL/R/390 - MS Rose 1976

Clinical studies indicate that man is more sensitive to the emetic effects of PP796 than experimental animals; emesis being seen at dose levels in the range 0.03-0.11 mg/kg (equivalent to total doses of 2-8 mg). In the first human study involving 12 healthy volunteers, 1 was given 0.25 mg, 1 was given 0.5 mg, 2 were given 1 mg, 3 were given 2mg, 2 were given 3 mg, 2 were given 4 mg and 1 was given 8 mg. Of these, the volunteer given 8 mg vomited, as did one of those given 4 mg. Almost all the volunteers reported nausea. When the blood levels of the 2 volunteers given 4 mg were compared, the one that vomited absorbed the compound more quickly. (As in dogs, the rate of absorption may be critical in determining vomiting.) With most of the volunteers complaining of nausea, vomiting, dizziness, sweating and flushing, PP796 is poorly tolerated at doses above 1-2 mg. As a consequence all further studies were conducted at the maximum dose of 2mg. (Of those that took 2 mg, approximately 10% vomited and 60% complained of nausea.)

From the limited data available in man is can be concluded that a dose of 5 mg should cause nausea and induce vomiting in the majority of those ingesting it. (Note – The clinical trials were conducted using PP796 in tablet form. This would have inevitably delayed absorption (Farrell 1970). (When present in a liquid pesticide formulation PP796 is generally in solution and may therefore be more readily absorbed.)

Perhaps a stronger database is available from deliberate ingestions of a liquid pesticide formulation in humans, when the ingested dose of emetic is calculated it results in the histogram given below. One hundred percent of those ingesting 0.2mg/kg PP796 or greater vomited within 30mins. (Produced from Meredith and Vale 1987)



Occupational exposure standard - OES112C - C Denman 1994
PP796 showed no protections against bronchospasm, no consistent effect upon blood pressure, no beneficial effect in psychiatric disorders, or on bodyweight in obesity and no effect on thyroid or adreno-cortical function. PP796 caused nausea, flushing, sweating, dizziness and vomiting at 1 mg doses and above. Angina pectoris was evident in 2/4 subjects following sub-acute dosing of 2 mg/kg, 3 times daily, after 4 and 6 weeks respectively; the effect ceasing on withdrawal of treatment. Capillary fragility with a positive Hess's test was noted in one subject. There was some limited evidence of acclimatisation to the initial nausea on repeat dosing. The half-life of PP796 in man following single oral doses (at levels between 0.25 and 8 mg) was between 1.5 and 3.5h.

MJL Clapp
27/02/04

References:

1. Farrell FG, (1970) ICI 63,197 Pharmacology and Biochemistry Volume II. ICI Pharmaceuticals Division Unpublished Report C2.10/01 RIC 4223

2. Farrell FG, (1970) ICI 63,197 Toxicity and Proposed Clinical Trials Volume III. ICI Pharmaceuticals Division Unpublished Report C2.10/02 RIC 4224 - includes
    i. TPR/194 Prolonged Toxicity tests (90day plus recovery) in the rat
    ii. TPR/64 Prolonged Toxicity tests (90day plus recovery) in the dog
    iii. The effect of ICI 63,197 on pregnancy and the foetus – Rabbit
    iv. The effect of ICI 63,197 on pregnancy and the foetus – Rat

3. Brammer, A; Robinson, M (1986) PP796: Emetic dose response study in dogs ICI Central Toxicology Laboratory Report No. CTL/T/2459

4. Brammer, A; Robinson, M (1986) PP796: Effect of stomach status on emetic efficacy in dogs. ICI Central Toxicology Laboratory Report No. CTL/T/2452

5. Case, D E; Dunlop, D (1977) The intravenous administration of ICI 63197 to beagle dogs. ICI Pharmaceuticals Division Report No. PH23517C

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13. Moses SE (1979) ICI 63,179 Skin and eye irritation, skin sensitisation potential and respirable fraction analysis. ICI Central Toxicology Laboratory Report No CTL/T/1277.

Please note reports highlighted in yellow are marked Company Secret Category 'C' reports