A new analysis of the human emetic dose-response to PP796 based on clinical data for dosing of PP796 only

Author

Kim Z Travis
Syngenta Fellow
Toxicology & Health Science
Syngenta Ltd
Jealott's Hill International Research Centre
Bracknell
Berkshire
RG42 6EY

Reviewer of biological interpretations and assumptions in this analysis

Richard Lewis
Global Head of Toxicology & Health Science
(same address as above)

Conductor and reviewer of the statistical dose-response analysis

Eddie McIndoe – Senior Biometrician
Keith Ward – Head of Biometrics & Syngenta Fellow
(same address as above)

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Introduction

PP796 is the code most commonly used to refer to 2-amino-4,5-dihydro-6-methyl-4-propyl-s-triazolo(1,5-alpha)pyrimidin-5-one, which is an emetic used in paraquat dichloride technical material and paraquat-containing formulations, in order to reduce the severity of human oral poisoning through accidental drinking of paraquat-containing formulations through induction of vomiting. The emetic is routinely included in paraquat dichloride technical at the point of manufacture as few formulation sites have the necessary emetic handling facilities to add at the point of formulation production. This molecule, as a phosphodiesterase inhibitor, was invented in the 1960s by ICI (Pharmaceuticals Division), a Syngenta predecessor company. In the 1960s it was known as ICI 63,197 and a number of small-scale clinical trials were started in healthy volunteers and patients with a variety of medical conditions. It was soon found to cause nausea, vomiting, dizziness and flushing at relatively low doses, and the clinical trial program was brought to an end. The inclusion of the PP796 emetic in paraquat products was one of several measures which ICI voluntarily adopted from 1977 onwards to reduce the frequency and severity of incidents of accidental drinking of paraquat-containing products. These measures also included the use of a dye and an odour in paraquat products to distinguish them from beverages, increased training for applicators and labelling emphasizing the importance of not placing paraquat into drink or other containers.

The purpose of this report is to revisit the ICI Pharmaceuticals human data on the emetic effect of PP796 alone, and to re-assess the dose-response.

Inclusion level of PP796 in paraquat

The amount of PP796 to be included in paraquat-containing formulations is clearly a matter of some importance. The original decision on the PP796 inclusion level was based on the recommendations of Rose (1976), in an analysis issued as an internal ICI Central Toxicology Laboratory, CTL (R, Research) report which analysed the limited ICI Pharmaceuticals data on the emetic effect of PP796 alone. Following the introduction of PP796 into paraquat formulations, work was conducted to assess the emetic response in individuals drinking paraquat formulations (containing paraquat, PP796, stench, dye and other co-formulants). These human poisoning incident data were published (Meredith & Vale, 1987). The publication reports that, overall, 65% of those drinking a paraquat formulation containing the emetic vomited within 30 minutes and, with respect to accidental poisoning where lower volumes were ingested, 55% of those consuming < 2 g paraquat ion (equivalent to approximately 10 mL of the standard global 'Gramoxone' formulation containing 200 g paraquat ion/litre) vomited within 30 minutes. It is this published human poisoning data which supports the current 'emetic clause' in the FAO specification, i.e. "*Emesis must occur in about half an hour in at least 50% of cases*". In full, the current FAO specification (FAO, 2008) states:

"An effective emetic, having the following characteristics, must be incorporated into the TK/SL/WG[1].
- It must be rapidly absorbed (more rapidly than paraquat) and be quick acting. Emesis must occur in about half an hour in at least 50% of cases.
- It must be an effective (strong) stimulant of the emetic centre of the brain, to produce effective emesis. The emetic effect should have a limited 'action period', of about two to three hours, to allow effective treatment of poisoning.
- It must act centrally on the emetic centre in the brain.
- It must not be a gastric irritant because, as paraquat is itself an irritant, this could potentiate the toxicity of paraquat.
- It must be toxicologically acceptable. It must have a short half-life in the body (to comply with the need for a limited action period).
- It must be compatible with, and stable in, the paraquat formulation and not affect the herbicidal efficacy or occupational use of the product.
To date, the only compound found to meet these requirements is 2-amino-4,5-dihydro-6-methyl-

4-propyl-*s*-triazole-(1,5*a*)pyrimidin-5-one (PP796)."

[1]The same wording is used for the specification of the technical concentrate (TK), soluble concentrate (SL) and water soluble granules (SG).

The human clinical data for PP796 alone

The current inclusion level of PP796 is supported by the human poisoning incident data in which people drank paraquat formulations including PP796. This is the most relevant data to use for this purpose, and provides support by demonstrating that the original decision on the inclusion level, based on the original estimate by Rose (1976), was reasonable. However, questions have recently been raised about the analysis reported in Rose (1976). Specifically, the assessment of the human dose-response in that report has been questioned. Therefore, the ICI Pharmaceuticals data on the emetic effect of PP796 alone are revisited here. This review goes back to the original ICI Pharmaceuticals Division human clinical data in order to conduct an entirely new analysis.

The human clinical data on PP796 (then called ICI 63,197) were originally collated in Bayliss (1973) and are summarised below, focussing specifically on the emetic response.

Trial (location/investigator)	Trial code[1]	Subjects	Dose Regimen	n	n vomited (time of vomiting)	Average body weight
Dundee/Crooks	A	Fit students	1 x 0.25 to 8 mg[2]	12	2 (30' & 120')	69 kg[3]
Manchester/Davies #1	B	Fit students	1 x 2 mg	8	1 (45')	no data, assume 70 kg
Manchester/Davies #2	C	Fit volunteers	2 x 2 mg with 7 day interval between doses	2	0	no data, assume 70 kg
Glasgow/Kerr	D	Allergic asthmatic 18-45 years	1 x 2 mg	4	1 (no time stated but probably within 150')	no data, assume 70 kg
Aberdeen/Palmer[4]	E	Asthmatic 18-45 years	2 x 2 mg with unspecified interval between doses	1	0	71 kg
Aberdeen/Palmer[4]	E	Asthmatic 18-45 years	1 x 2 mg	1	1 (20')	70 kg
Utrecht/Beumer	F	Mild to moderate emphysema	single unspecified dose	12	not recorded	no data
Edinburgh/Eccleston	G	Classic endogenous depression 18-60 years	21 days of 3 x 2 mg each day	4	0	no data, assume 123kg[5]
Birmingham/Magnus #1	H	Chronic inpatient schizophrenia	7 days of 3 x 1 mg each day, then 7 days of 3 x 2 mg each day	6	0	134 kg
Birmingham/Magnus #2	I	Anxiety 18-50 years	8 days of 3 x 1 mg each day, then 7 days of 3 x 2 mg each day	5	1 (no time stated, but whilst on 2mg doses)	126 kg
Bebington/Zacharias	J	Sustained diastolic hypertension 18-60 years	28 days of 4 x 2 mg each day	3	0	77 kg
Manchester/Davies #3	K	Obesity 30-55 years	42 days of 3 x 2 mg each day	4	0	123 kg

[1] This code is used in the next table to indicate from which trial(s) each datapoint on the dose-response came.

[2] In the trial conducted by Crooks in Dundee, each person received a single dose of PP796, the dose levels and numbers of people being 0.25 mg (1 person), 0.5 mg (1), 1 mg (2), 2 mg (3), 3 mg (2), 4 mg (2), and 8 mg (1).

[3] For the dose-response analysis below, the average body weights of each individual dose group in this study are used.

[4] In the trial conducted by Palmer at Aberdeen, one patient was given the intended two doses, but dosing was stopped for the other patient because they vomited after the first dose. Since the dose regimens differ for these two patients they are placed in separate rows.

[5] For the trial conducted by Eccleston in Edinburgh no body weight data are available. These patients had classic endogenous depression, which is strongly correlated with being overweight (*e.g.* Luppino *et al*, 2010). Therefore higher than typical body weight is assumed for this group, specifically 123kg, which enables the data to be combined with those from Davies's trial of obese patients in Manchester. For all other groups for which body weight data was not available, 70 kg was assumed.

The trial conducted by Beumer in Utrecht was more poorly documented than the rest. Specifically, the dose level was not stated, and it is also not clear that if emesis had occurred then it would have been recorded and included in Bayliss (1973). For these reasons this trial is excluded from this analysis.

Although the clinical trials were conducted in healthy volunteers and in patients with a variety of clinical conditions, it was concluded that none of these clinical conditions were likely to have affected the emetic response of the individuals. Therefore no groups were excluded from the dose-response analysis based on clinical condition. Reported conditions likely to have affected emetic response or gastrointestinal absorption would have resulted in their exclusion, *e.g.* Crohn's disease.

When evaluating the dose-response of PP796 based on these data, it would be ideal to use data where single doses were given. A single dose relates to the accidental poisoning incidents for which the emetic was intended to have a beneficial effect. However, several of the clinical trials listed above included multiple dosing occasions. Available data on the human kinetics of PP796 (Bayliss, 1973) is not adequate to support a sophisticated analysis of the relationship between blood or plasma concentrations of PP796 and emesis over multiple doses. Therefore the approach taken here is to consider only the first dose of PP796 that each person in each clinical trial received, and whether this did or did not result in emesis. This enables both single dose and multiple dose clinical trials data to be used in the dose-response analysis.

An alternative and inappropriate approach to the analysis of the multiple dose clinical trials data is illustrated using the following example. Consider one of the patients in the trial performed by Zacharias at Bebington, who took a 2 mg dose of PP796 four times a day for 28 days, 112 doses in all, and did not vomit. It would <u>not</u> be valid to consider this patient to represent 112 occasions in which a 2 mg dose of PP796 failed to result in vomiting, and incorporate all 112 occasions into the overall dose-response assessment as independent observations. These observations are not independent because they are for the same patient. It is also clear from the limited human kinetic data available (Bayliss, 1973) that PP796 will not all be cleared from the body between successive dosing occasions, making such an analysis even more problematic. Despite the likelihood that the systemic concentration of PP796 would be expected to increase with multiple doses, this did not result in a marked increase in vomiting with multiple compared to single doses in this series of clinical trials. Indeed, in the trial by Eccleston at Edinburgh it was noted that patients receiving 2 mg of PP796 three times a day for 21 days experienced nausea for the first 3-5 days, which subsequently "*wore off with no intervention*". This is suggestive of an adaptive response, which if it occurred would further invalidate the consideration of emesis after multiple doses when estimating the dose-response of a single dose of PP796.

The timing of emesis is important, as emesis of a paraquat formulation very soon after drinking will tend to clear more paraquat from the body than later emesis. This is encapsulated in the 'emetic clause' in the FAO specification, i.e. "*Emesis must occur in about half an hour in at least 50% of cases*". The FAO specification reflects the desirable speed of emesis, *i.e.* achieving emesis within 30

minutes. However, less was known about the necessary speed of emesis in man in 1976. Rose (1976) recognised the benefit of emesis occurring within one hour and targeted this as the objective. In considering the human clinical data he considered only whether people did or did not vomit, irrespective of the timing of emesis, but he did speculate that emesis would likely be faster if PP796 were present in a paraquat formulation in comparison to the clinical trials of PP796 because:

- the clinical trials used PP796 tablets, whereas PP796 dissolved in formulation would be absorbed more quickly;
- the irritancy of paraquat dichloride might reasonably be expected to accelerate emesis compared to dosing PP796 alone.

To allow for the various different perspectives on the necessary speed of emesis, in the current statistical analysis three dose responses will be considered:

- Vomiting at any time
- Vomiting within one hour
- Vomiting within 30 minutes

The trial conducted by Kerr at Glasgow needs careful handling in this context. Of the four patients in this trial, only one vomited. These patients were observed in detail for 150 minutes (Bayliss, 1973), and it is therefore likely that vomiting occurred within this period, but it is not known if vomiting was within 30 minutes or within one hour of dosing. It has been decided to deal with the data from this particular trial as follows: for the analysis of the dose response for vomiting at any time, the data will be used, but for the analysis of the dose responses for vomiting within one hour or within 30 minutes the data from this entire trial will be excluded (the data for the three patients who did not vomit must also be excluded or else it would result in a biased analysis).

Based on the above data handling considerations, and combining groups which received the same (first) dose level of PP796 and had average body weights within 10% of each other, the following summary table can be produced. The dose of PP796 expressed in units of mg of PP796 per kilogram of body weight are used for the dose-response analysis, as is standard best practice in toxicology.

Dose (mg)	Body weight (kg)	Dose (mg/kg)	n	n (vomited)	n (vomited within one hour)	n (vomited within 30mins)	Data source[1]
0.25	50.5	0.0050	1	0	0	0	A
0.5	77.5	0.0065	1	0	0	0	A
1	130	0.0077	11	0	0	0	H,I
1	70	0.014	2	0	0	0	A
2	123	0.016	8	0	0	0	K
2	70	0.029[2]	19	3			E,J,B,C,D
2	70	0.029[2]	15		2	1	E,J,B,C
2	55.8	0.036	3	0	0	0	A
3	75.5	0.040	2	0	0	0	A
4	81.2	0.049	2	1	1	1	A
8	80	0.10	1	1	0	0	A

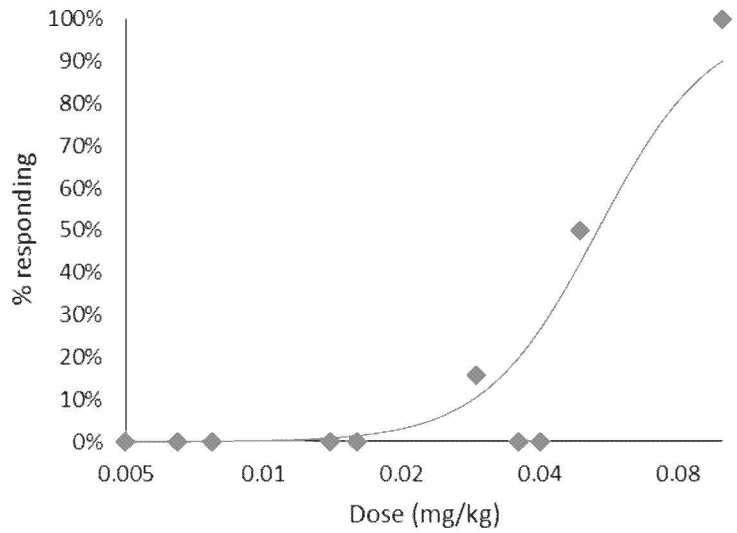
[1] This column corresponds to the 'Trial code' column in the previous table, and indicates from which trial(s) each datapoint on the dose-response came.

[2] At this dose level the trial conducted by Kerr at Glasgow with four patients (trial code D) is included for the analysis of vomiting at any time, but is excluded from the analysis of vomiting within one hour or within 30 minutes, as explained in the text above.

Dose-response analysis for emesis

The ED50 for emesis, i.e. the dose resulting in a 50% probability of causing emesis, was estimated by regression analysis carried out using PROC PROBIT in SAS Software, version 9.4, assuming a binomial error structure and a logit link function. The dose response for vomiting at any time was successfully fitted. However, it was found that the dose responses for vomiting within one hour or within 30 minutes do not allow the establishment of a dose response relationship and are therefore not capable of yielding an ED50 value. This is largely because the one person receiving an 8mg dose vomited at 2 hours, and so 100% vomiting response at this dose becomes 0% when considering vomiting which occurs within one hour or 30 minutes.

The dose-response data and the best-fit dose-response curve for vomiting at any time are shown below.



The best estimate of the dose resulting in a 50% chance of vomiting at any time is 0.053 mg/kg (95% confidence intervals are 0.038-39857). Expressed in mg, assuming a 70kg body weight, the best estimate of the dose resulting in a 50% chance of vomiting at any time is 3.7 mg (95% confidence intervals are 2.7-2790022). There was no statistical evidence of a lack of fit of the logit regression curve to the data, i.e. the deviation of the data points around the fitted curve are consistent with random binomial variation.

Discussion

The human clinical data for the emesis caused by PP796 alone are not ideal for defining a dose-response. There are no dose groups of any significant size for which half or more of the people vomited. This is reflected in the very wide confidence intervals for the dose resulting in a 50% probability of vomiting at any time. At the second highest dose one out of two people vomited and

at the highest dose one out of one vomited. These datapoints are suggestive of a steep dose-response (see figure above), but the tiny numbers of people involved mean that these datapoints are highly uncertain and the fitted dose response is therefore also highly uncertain. The data for vomiting within an hour or within 30 minutes are weaker still, and do not support the fitting of a dose-response relationship.

The decisions taken and documented here as part of this dose-response analysis differ from those taken by Rose (1976). Rose did not document the rationale for the decisions he took in converting the data in Bayliss (1973) into a summary tabular form for dose-response analysis. In estimating the emetic dose in man Rose clearly recognised that for the emetic dose response to PP796 there was "*limited data available in man*". He seems to have visually inspected the data and drawn his conclusions, rather than using any statistical procedure. Rose concluded that "*a dose of 5mg should certainly cause nausea and ought to induce vomiting in the majority of those ingesting it*". This estimate of an effective emetic dose of 5mg is close to the best estimate of 3.7mg from the current analysis for the dose resulting in a 50% probability of vomiting at any time. To this extent, despite the many differences in approach, the two analyses produce a similar best estimate of the effective PP796 emetic dose. However, the great uncertainty demonstrated by the wide 95% confidence intervals in the current analysis (2.7 to 2790022 mg) means that the clinical data are incapable of supporting a confident conclusion on the appropriate inclusion level of PP796 in paraquat formulations. When considering the dose needed to achieve emesis within one hour, or within 30 minutes, the uncertainties increase further. The lack of adequate consideration of uncertainty is very common in the scientific community, and was even more common in the past. Indeed, it is only a proper statistical analysis of the dose-response data which fully reveals the uncertainties, and there is no evidence of any statistical approach being used in Rose (1976). Anyone not properly considering statistical uncertainties might analyse these data and come to similar conclusions to those in Rose (1976).

The Rose (1976) analysis was instrumental in determining the current standard inclusion level of PP796 in paraquat formulations originally estimated to result in emesis within one hour in the majority of individuals ingesting a minimal lethal dose. The retrospective published analysis of human paraquat poisoning incidents in Meredith & Vale (1987) showed that this inclusion level was effective in achieving emesis within 30 minutes for those consuming <2 g paraquat ion (equivalent to approximately 10 mL of a standard 200 g paraquat ion/litre formulation).

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