Phenylbutazone in horses and man: Properties relevant to safety of humans consuming horse meat containing phenylbutazone and its metabolites

Introduction
This editorial assesses the potential toxicity for human individuals consuming equine muscle containing phenylbutazone and its metabolites, in trace amounts, by reviewing: 1) the pharmacokinetic, pharmacodynamic, metabolic and toxicological profiles of phenylbutazone in horses and man; 2) phenylbutazone residues in equine muscle; 3) the extent of consumption of equine meat, containing phenylbutazone or not; and 4) the moves being made towards improving the equine passport system. This editorial summarises and extends the contents of a recent review (Lees and Toutain 2013).

Pharmacodynamics
(Lees et al. 2004)
Phenylbutazone is a nonsteroidal anti-inflammatory drug (NSAID). Its principal mechanism of action for both pharmacological and toxicological properties is inhibition of cyclooxygenase (COX). COX exists in two isoforms, COX-1 and COX-2. The former is a constitutive enzyme, present in most body cells; it subserves a range of cellular functions and its inhibition leads to several side effects. COX-2 is also constitutive in some organs and tissues, but it is additionally inducible at sites of inflammation, leading to the production of inflammatory mediators, such as prostaglandin E2.

Pharmacokinetics
The elimination half-life (t½) of phenylbutazone ranges from 1–2 h in the donkey, 4–6 h in the horse, 42–65 h in mature cattle, 50–105 h in man to 207 h in the neonatal calf (Table 1). In all species investigated, the volume of distribution is low (0.1–0.3 l/kg bwt) and clearance is very variable, ranging from very slow in the neonatal calf to very rapid in the donkey (Table 1).

Area under the plasma concentration-time curve (AUC) is a measure of overall exposure of body tissues to a drug, because it is the product of amount (concentration) and time. AUC is sometimes described as the internal (as opposed to administered/external) dose and is derived from the equation

\[ \text{AUC} = \frac{\text{Dose}}{\text{CT}} \times F \]

where F is another pharmacokinetic term, bioavailability; it is the amount [expressed as a fraction [from 0–1]] of the dose administered by a nonvascular route gaining access into the systemic circulation. From this relationship it follows that the internal exposure will be proportional to the bioavailability. The data in Table 1 indicate that phenylbutazone AUC is some 15–25 times greater in both mature cattle and humans than in the horse for a given dose rate. These differences are due mainly to species differences in plasma clearance.

The bioavailability of phenylbutazone in the horse, after oral dosing, is in the range of 70–90%. Moreover, absorption extent is only marginally affected by feed availability at the time of dosing. However, the absorption pattern is significantly altered. Thus, time to reach maximum concentration is 4–7 h when horses are deprived of access to feed for a few hours before and after dosing and is characterised by a single concentration peak. In contrast, when horses have free access to feed, 2 or 3 plasma concentration peaks are likely and maximum concentration is delayed to 10–18 h. A delayed absorption will therefore impact on the residue depletion profile (delayed by up to 12 h) in the case of oral dosing of phenylbutazone to fed horses.

General toxicological profile
(Snow et al. 1979; Lees et al. 1983; MacKay et al. 1983; Singh and Triadalifilopoulos 1999; Scarpignato and Hunt 2010) As a drug of the NSAID class, phenylbutazone exerts predictable dose-related side effects on liver and kidney and it inhibits blood clotting at high dose rates. Of particular concern, however, for most drugs of the NSAID group are the upper gastrointestinal side effects of perforation, ulceration and bleeding. In one early American study, an annual mortality rate of 16,500 from a NSAID-treated population of 13 million human individuals (incidence of 127/100,000/annum – see below for comparison with incidence rate of aplastic anaemia in phenylbutazone treated subjects) was estimated. Whilst the NSAID mortality rate in the 21st century is estimated to be much lower, these early figures emphasise the overall risk to humans taking NSAIDs chronically at recommended dose rates.

In the horse, phenylbutazone can be used with relative safety, when adhering to manufacturer’s recommended dose rates. However, its narrow safety margin should be recognised. Repeated daily oral or i.v. doses in the range of 8.8–30 mg/kg bwt have produced mortality related to protein-losing enteropathy, hepatotoxicity and renotoxicity. The daily dose should not exceed 4.4 mg/kg bwt and this should be reduced to 2.2 mg/kg bwt after a few days’ therapy.

Carcinogenicity
(Maekawa et al. 1987; Kani et al. 1995; Kirkland and Fowler 2010; Hall 2013)
In horses and man the recommended therapeutic dose rate of phenylbutazone is of the order of 2–5 mg/kg bwt daily. The significance to man of rodent carcinogenicity studies with phenylbutazone is questionable, given the heroically high dose rates used. One group concluded that there was evidence of carcinogenicity in male but not in female mice receiving doses of 150 or 300 mg/kg bwt 5 days per week for 2 years. Another group concluded that, at dietary incorporation levels of 0.25%, phenylbutazone exerted no carcinogenic activity in rats over a 2 year dosing period. The World Health Organization’s International Agency for Research on Cancer concluded that there was inadequate evidence of carcinogenicity.
TABLE 1: Species differences in clearance and elimination half-life of phenylbutazone*  

<table>
<thead>
<tr>
<th>Species</th>
<th>Clearance (mL/h/kg bwt)</th>
<th>Elimination half-life (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf (neonatal)</td>
<td>0.708</td>
<td>207</td>
</tr>
<tr>
<td>Human</td>
<td>Approximately 2</td>
<td>50–105</td>
</tr>
<tr>
<td>Cow</td>
<td>1.24–2.90</td>
<td>42–65</td>
</tr>
<tr>
<td>Goat</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>Camel</td>
<td>4.9–10.0</td>
<td>13</td>
</tr>
<tr>
<td>Horse</td>
<td>16.3–26.0</td>
<td>4.0–6.0</td>
</tr>
<tr>
<td>Dog</td>
<td>18.4</td>
<td>2.0–6.0</td>
</tr>
<tr>
<td>Rat</td>
<td>35–83</td>
<td>2.8–5.4</td>
</tr>
<tr>
<td>Pig</td>
<td>39</td>
<td>1.83</td>
</tr>
<tr>
<td>Rabbit</td>
<td>65</td>
<td>1.89</td>
</tr>
<tr>
<td>Donkey</td>
<td>170</td>
<td>1.0–2.0</td>
</tr>
</tbody>
</table>

*Data from various publications (summarised in Lees et al. 2004).

of phenylbutazone in man (IARC Monographs 1977). Putting these data in context, a recent review pointed out that the average American ingests daily 0.09 mg of synthetic pesticide residues in food together with some 1500 mg of naturally occurring pesticides. Many of the latter have tested positive in carcinogenicity studies. The same reviewer noted that coffee contains 14 compounds that cause cancer in rodents. It has furthermore been explained that the theoretical carcinogenic effects of phenylbutazone in man cannot be studied, because patients prescribed the drug were given doses far below the level any effect may become apparent (<1 mmol/l, i.e. <308 mg/l).

Blood dyscrasias in man

(von Rechenberg 1962; Fowler 1967; Bottiger and Westerholm 1973; Ramsey and Golde 1976; Smith et al. 1977; Chaplin 1986; Faich 1987)

Restrictions on the use of phenylbutazone in man in the 1980s resulted from the recognition that it can induce blood dyscrasias, including aplastic anaemia, leukopenia, agranulocytosis and thrombocytopenia, in some cases leading to death. Fatalities were associated with use at the manufacturer’s recommended dose rate, generally after therapy over several weeks or months. Nevertheless, therapeutic use was still allowed in individual patients, under specialist control and with regular blood profile monitoring. In some countries, phenylbutazone licensed by generic companies has continued to be used to this day in human medicine.

Von Rechenberg (1962) reported 51 deaths and 38 nonfatal cases of agranulocytosis linked to phenylbutazone usage from an estimated population of 50 million treated human patients. In the early period of phenylbutazone usage (commonly with the high dosage of 1 g [or higher]/subject/day), fatal blood dyscrasias were reported in 133 human patients, comprising an incidence of 1 in 20,000 treated patients/annum. Subsequent surveys indicated that deaths from aplastic anaemia occurred in the UK with an incidence of ~1 in 40,000–50,000 phenylbutazone treated patients.

In a 1984 FDA analysis of NSAID-associated fatalities attributable to haematological conditions, the highest percentage was for phenylbutazone (54%), followed by ibuprofen (24%), fenoprofen (23%) and naproxen (0%). Therefore, haematopathology-based fatalities are not unique to phenylbutazone and other pyrazolones. Similar estimated fatality rates of 15–20 per million exposures for: 1) phenylbutazone and aplastic anaemia; 2) aspirin and gastrointestinal bleeding; and 3) penicillin-induced anaphylaxis have been made.

Based on in vitro studies designed to elucidate the mechanism(s) of phenylbutazone-induced blood dyscrasias, several groups have demonstrated concentration dependency of myelotoxicity. It has also been reported, from clinical use of phenylbutazone, that ‘although the mechanism of its haematologic toxicity is not known, there is some evidence that it is dose related. The incidence of leucopenia, thrombocytopenia, anaemia and aplastic anaemia, in the earliest clinical trials using doses up to 1.6 g per subject daily, was considerably higher than later trials using lower dosages.’ (Ramsey and Golde 1976).

The possibility of rare individual sensitivity, even to very low phenylbutazone/oxyphenbutazone concentrations, cannot be excluded: it is not possible to prove a negative. However, the available human data relate to rare sensitivity to full therapeutic doses. Even if the sensitivity, as well as being very rare, were to occur at low concentrations, there must be a limiting lower concentration and period of exposure, below which this type of toxicity will not occur. The epidemiological finding of medium (several weeks) to long (several months) median daily exposures, prior to the occurrence of agranulocytosis and aplastic anaemia, respectively, points towards a likely dependence on daily dose and duration of dosing.

Hazard and risk

Hazard and risk are different concepts. Hazard is the intrinsic capacity of a substance to cause harm (e.g. phenylbutazone causes aplastic anaemia in humans) whereas risk depends on the situation in which the hazard may be expressed. Thus hazard is constant, whilst risk changes with circumstances, including exposure. Risk depends on the route of exposure, whether it is systemic or local, the magnitude of exposure (e.g. the external dose in mg/kg bwt and the internal dose expressed as plasma AUC), frequency of exposure and susceptibility of the exposed group.

Horse meat consumption in the EU

In 2008 the known horse meat consumption in the EU was 96,000 tonnes, comprising 0.2% of total meat consumption, involving an average yearly meat consumption of ~170 g/inhabitant. Countries with the highest consumption are Italy (45,000 tonnes in 2008, corresponding to 1% of the total domestic Italian meat consumption) and France (20,000 tonnes in 2008, comprising 0.4% of the total domestic meat consumption). In France, horse meat consumption in 2011 was 17,967 tonnes, providing a nominal average meat consumption per capita of ~300 g. The concept of an average consumer for horse meat is inappropriate, however, as horse meat is consumed by a small fraction of the population. In France, 16.7% of homes purchased horse meat in 2011, so that consumption up to 10 kg/year is to be expected in the highest consumers.

Residues depletion

(Toutain et al. 1980a,b; Lees et al. 1987)

From the pharmacokinetic profile of phenylbutazone in the horse, it is predicted that some 75% of the drug in the body is...
located in extracellular fluids (including 29% in plasma) and only 25% is present in the remainder of the body. As the volume of intracellular fluid exceeds that of extracellular fluid, it is further predicted that tissue concentrations of phenylbutazone will be relatively low. Rates of depletion from edible tissues are parallel to plasma depletion rate and can be readily predicted from knowledge of the plasma concentration-time profile. In an equine study, conducted in a limited number of animals, phenylbutazone plasma: tissue concentration ratios were in the ranges 6:1 to 25:1 (muscle), 32:1 to 5:1 (liver) and 6:1 to 3:1 (kidney cortex). Consequently, muscle concentrations were very low (100–200 μg/kg, that is 100–200 ng/g) within 12 h of oral dosing of phenylbutazone at the recommended dose rate of 4.4 mg/kg bwt. In another equine study, after intravenous dosing at the high dose rate of 8.8 mg/kg bwt, plasma concentrations had decreased to 1.24 and 0.16 mg/l, respectively, at 24 and 48 h. Assuming a plasma: muscle concentration ratio of 30:1, predicted muscle concentrations would be low, 41 and 5 μg/kg, respectively, at 24 and 48 h.

Broadly similar plasma: muscle concentration ratios of phenylbutazone have been reported experimentally in other species, 10:1 in the dog, 8:1 to 21:1 in cattle and 16:1 to 33:1 in the rabbit. Consideration should also be given to hydroxylated metabolites of phenylbutazone, notably oxypHENbutazone and γ-hydroxyphenylbutazone, as both are pharmacologically (and potentially toxicologically) active. However, in the horse, plasma concentrations of γ-hydroxyphenylbutazone are very low and those of oxypHENbutazone of the order of 20% of phenylbutazone concentrations. Equine muscle concentrations of oxypHENbutazone were low (100 μg/kg) 6 h after oral or i.v. dosing of phenylbutazone at a dose rate of 4.4 mg/kg bwt.

In summary, concentrations of phenylbutazone and its metabolites in equine muscle are at all times low after administration of therapeutic doses and, beyond 24 h after dosing, concentrations are in parts/billion and decreasing.

Detection and quantification of phenylbutazone in horse muscle and processed meats

In the UK, the level of quantification for phenylbutazone is 0.6 μg/kg and the level of detection is ~0.4 μg/kg. The target tissue is kidney and a sample is considered to be positive (traces) in the concentration range 0.4–0.6 μg/kg and positive (quantified) >0.6 μg/kg. Approximately 8000 horses are slaughtered annually for human consumption in the UK. The majority are exported to other EU countries. In 2011, 1,681 (1.9%) of kidney samples taken at abattoirs tested positive for phenylbutazone (http://www.vmd.defra.gov.uk/VRCPdf/PositionPaper_Phenylbutazone.pdf). In 2012, 8426 horses were slaughtered for human consumption and, of these, 145 kidney samples were tested for phenylbutazone; 5/145 (3.44%) tested positive. Multiplying this figure for all slaughtered animals, gives an estimated incidence rate of 290 for the 8426 horses. The UK Foods Standards Agency (FSA) reported that, between 30 January and 7 February 2013, 206 equine carcasses had been tested for phenylbutazone; of these 8 (3.9%) tested positive (http://www.food.gov.uk/news-updates/news/2013/feb/bute-carcasses-results#.UW9hcZFO.jo).

Based on EU National Residue Control Plans, an April 2013 report (EFSA/EMA, 2013) for 19 member states indicated that 37 (1.55%) of 2386 horse tissue samples were positive for phenylbutazone. The highest detection rate was for kidney (2.8%); the highest concentration reported was 1900 μg/kg but the median kidney concentration was low, 4.0 μg/kg. Of 672 muscle samples analysed, one (0.1%) was positive for phenylbutazone at a concentration of 19.2 μg/kg.

These data provide cause for some concern, as phenylbutazone is banned for use in food producing species in all EU countries. The range and mean of concentrations detected are not always provided in these reports. However, this is important, as it might or might not alleviate concerns on residues, noting particularly that muscle concentrations will normally be significantly lower than those in kidney. A new monitoring system, introduced on 30 January 2013, involves the testing of all horse carcasses for phenylbutazone in the UK. Only those testing negative will be released from the slaughterhouse. Test results become available within ~48 h.

**Intake of phenylbutazone from horse meat and processed meat products**

(Laporte et al. 1998; Orszag 2008)

Based on actual or estimated data, a range of calculations can be undertaken to determine the consumption of phenylbutazone from eating horse meat. Three examples will be considered. 1 After an intravenous high dose of phenylbutazone (8.8 mg/kg bwt), mean plasma concentrations at 24, 48 and 72 h were 1.24, 0.16 and 0.057 mg/l, respectively. If a plasma-to-muscle concentration ratio of 30:1 is assumed and, based on meat intake of 300 g, the amount of phenylbutazone ingested by a 70 kg human would be 12.4, 1.6 and 0.57 μg in total at these times. This constitutes a very much lower exposure than the recommended human daily therapeutic dose [2–5 mg/kg bwt, corresponding to 140,000–350,000 μg in total for a 70 kg person]. 2 Assuming a commercial burger weights 100 g and, assuming further that an individual consumes at one sitting one burger containing 100% horse meat, and also assuming the horse was slaughtered 12 h after receiving a therapeutic dose rate of 4.4 mg/kg bwt, the quantity of phenylbutazone ingested would be of the order of 20 μg for a 40 kg child, corresponding to a phenylbutazone ‘dose’ of 0.5 μg/kg bwt bodyweight. This can be compared with the recommended daily dose of phenylbutazone for human use of 2000–5000 μg/kg bwt. 3) Considering the scenario that all slaughtered horses, destined for consumption of their meat, received a maximum recommended phenylbutazone dose 24 h before slaughter; the annual exposure to phenylbutazone for a heavy horse meat consumer (10 kg per year) would not exceed 1200 μg. This corresponds to 17 μg/kg bwt for an adult of 70 kg weight, which is of the order of 1/200th of a single daily therapeutic human dose. These examples of low ‘intake doses’ can be regarded as ‘worst case’, because: 1) many horses will not be slaughtered within 24 h of dosing; and 2) derived products might contain less than 100% horse meat. On 15 February 2013, the UK Food Standards Agency (FSA) reported that, of 2501 ‘beef’ products tested, 29 were positive for horse DNA: of these 29 samples, all tested negative for phenylbutazone.

These hypothetical examples of ingestion of phenylbutazone comprise amounts that are massively below any possible pharmacological or general toxicological effects. However, the risk of blood dyscrasias should be considered separately. Chloramphenicol was banned from use in food producing species in the EU, because of a rare but potentially fatal risk of causing aplastic anaemia in humans.
However, it was shown that the reaction could occur with exposure to very small amounts, thus explaining a possible association between the administration of topical ocular chloramphenicol and aplastic anaemia. Therefore, very low concentrations of chloramphenicol may constitute a potential hazard, when present in foodstuffs. In contrast, the balance of evidence is that blood dyscrasias caused by phenylbutazone are both idiosyncratic and dose-related. Furthermore, the incidence of life-threatening blood dyscrasias is of the order of 1 in 20,000–100,000 for therapeutically exposed human subjects (2–6 mg/kg bwt daily, generally for several weeks or months). Moreover, the lack of detectable concentrations of phenylbutazone, using analytical assays with an ability to detect concentrations in kidney of ∼0.6–5 μg/kg, in a high proportion of horse tissue samples should be noted. Hence, the likelihood of a blood dyscrasia attributable to a phenylbutazone residue arising in an EU consumer of horse meat is vanishingly small, particularly when the management options (e.g. EU ban on phenylbutazone in food producing species, passports for horses) are revised and made to operate more effectively.

European Union regulations to guarantee the safety of horse meat for human consumers

Whilst phenylbutazone is not banned from equine use, it is banned from use in any food producing animal. Within the EU, management protocols are designed to prevent slaughtered horses, treated in life with phenylbutazone, from entering the food chain. Equids must be accompanied by an individual passport during their movements. Moreover, all medicinal treatments must also be noted in records kept on farms in the UK (where the horse is not a food producing animal), but can potentially enter the EU food chain following export). Document VMGN16 (http://www.vmd.defra.gov.uk/public/vmr_vmgm.aspx) describes the circumstances and procedures relating to the use of all medicines in horses and how the passport system is intended to operate. Moreover, ‘passports must be signed at part II of section IX to indicate that the animal is not intended for human consumption’. This is a legal responsibility under the UK Horse Passport regulations described on the website (Note 16 http://www.vmd.defra.gov.uk).

A UK Veterinary Residues Committee position paper (http://www.vmd.defra.gov.uk/VRC/pdf/PositionPaper_Phenylbutazone.pdf) indicated that some veterinary surgeons were prescribing phenylbutazone for horses without checking the passports or ensuring that the animals were subsequently excluded from the food chain. Moreover, phenylbutazone residues have been detected in horses that have changed owners prior to slaughter and whose passports did not indicate that they have been signed out of the food chain. Even when a horse has been exported, the FSA informs the European Commission in the event of a positive.

Recent correspondence indicates that, following review, the many deficiencies in the current passport system (including poor understanding and poor compliance) are to be addressed, with a view to ensuring minimal opportunity for meat from phenylbutazone-treated horses entering the human food chain (Jones and Chandler 2013: Rowlords 2013; Veterinary Record Editorial 2013: Westgate 2013). On passports, the European Commission is currently consulting on reform of the equine identification regulations, to include making a national database compulsory and reducing the number of passport issuers (http://ec.europa.eu/food/food/horsemeat/plan_en.htm).

Joint statement of EFSA and EMA (2013)
The European Food Safety Authority and EMA recently issued the following statement: ‘on the basis of limited monitoring data in 19 member states . . . on a given day, the probability of a consumer being both susceptible to developing aplastic anaemia and being exposed to phenylbutazone was estimated to range approximately from 2 in a trillion to 1 in 100 million’. Fine, but what this report does not address are the additional risk factors discussed in this editorial, including the all important exceedingly low levels of exposure to phenylbutazone and its metabolites from horse meat consumption. As we emphasise, the evidence for blood dyscrasias in response to phenylbutazone relate to therapeutic use of high doses administered daily for several weeks or months.

Authors’ declaration of interests

No conflicts of interest have been declared.

P. LEES and P.-L. TOUTAIN*
Royal Veterinary College, Hatfield, UK; and *Ecole National Vétérinaire de Toulouse, France.

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