

Joint Statement of EMA and EFSA on the presence of residues of phenylbutazone in horse meat

European Food Safety Authority (EFSA), Parma, Italy

European Medicines Agency (EMA), London, United Kingdom

Abstract

Controls in Member States have revealed the presence of phenylbutazone in horse carcasses intended for the food chain. Following the request from the European Commission, the European Medicines Agency and the European Food Safety Authority jointly concluded on the risk assessment on residues of phenylbutazone in horse meat in the context of recent fraudulent practices. The Committee for Veterinary Medicinal Products assessed the consumer safety for phenylbutazone in 1997 and identified the main risks for the consumer as idiosyncratic¹ blood dyscrasias and the genotoxic/carcinogenic potential for which no thresholds could be identified and no maximum residue limits could be established. The substance can therefore not be used in animals destined to enter the food chain. These main risks have been re-confirmed in the present statement as no new relevant information has become available since the initial safety assessment. Exposure to phenylbutazone from horse meat consumed as such or present in beef-based products was assessed on the basis of limited monitoring data provided by 19 Member States and conservative assumptions. Up to 144 and up to 36,800 individuals per 100 million could be potentially exposed across countries and age groups each day. 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. The risk of carcinogenicity to humans from exposure was considered very low based on the available experimental data on organ toxicity and carcinogenicity, as well as on the low exposure levels and the infrequent exposure to phenylbutazone from horse meat or adulterated beef-based products. Measures proposed to further minimise the risk include strengthening of the horse passport system, harmonised monitoring of phenylbutazone and its main metabolite and better reporting of monitoring of veterinary drug residues and other substances across the EU

Key words

phenylbutazone, oxyphenbutazone, horse meat

¹ An idiosyncratic reaction is an unusual reaction to drugs, i.e. only expressed by some individuals.

See websites for contact details

Table of contents

1. Introduction	4
2. Hazard identification and characterisation	4
2.1. Safety assessment	5
2.1.1. Blood dyscrasias in humans	5
2.1.2. Mutagenicity and genotoxicity	6
2.1.3. Carcinogenicity	6
2.1.4. Reproductive toxicity	7
2.1.5. Subchronic and chronic toxicity	8
2.2. Conclusions on hazard identification and characterisation	8
3. Exposure assessment	9
3.1. Prevalence of phenylbutazone in horse samples	9
3.2. Prevalence of beef-based products adulterated with horse meat	9
3.3. Likelihood of dietary exposure to phenylbutazone	10
3.4. Levels of exposure to phenylbutazone	10
4. Risk characterisation	11
5. Uncertainty analysis	11
Conclusions and recommendations	13
Conclusions	13
Recommendations	14
References	16
Appendices	18
Abbreviations	51

Background as provided by the European Commission

Phenylbutazone (4-butyl-1,2-diphenyl-3,5-pyrazolidinedione) is a non-steroidal anti-inflammatory drug (NSAID) for the short-term treatment of pain and fever in animals.

Phenylbutazone is not authorised for use in food-producing animals as maximum residue limits are not established for the substance. In the EU several human medicinal products with the pharmacological substance phenylbutazone have a marketing authorisation.

Controls in Member States have revealed the presence of residues of phenylbutazone in horse meat which indicate an illegal use of carcasses of horses treated with this pharmacological substance.

The presence of residues of phenylbutazone in horse meat prompted the competent authorities to take appropriate and proportionate actions as laid down in Directive 96/23/EC. These include, amongst others, restrictions on movement of animals and obligatory testing of the remaining animals.

Terms of reference as provided by the European Commission

The European Commission asks the European Food Safety Agency and the European Medicines Agency for a joint statement on the presence of residues of phenylbutazone in horse meat.

The Agencies shall perform a risk assessment on residues of phenylbutazone in horse meat.

The statement shall provide advice on any potential risk posed to consumers from the presence of residues of phenylbutazone in horse meat. In evaluating the matter, the agencies should consider both the risk posed from direct consumption of horse meat and the risk from other products illegally contaminated with such food.

The joint statement should identify, where appropriate, if additional control options are needed to minimise the risks identified.

Approach taken to answer the terms of reference

European Medicines Agency (EMA) and European Food Safety Authority (EFSA) set up a joint ad-hoc expert group for the preparation of the Scientific output. Experts from the EFSA Panel on Contaminants in the Food Chain, experts on safety from the EMA Committee for Medicinal Products for Veterinary Use (CVMP) and the EMA Committee for Medicinal Products for Human Use (CHMP) were nominated as members of the joint expert group. EFSA was responsible for the exposure assessment and EMA was responsible for the safety assessment. The overall risk assessment was finalised by the full group of experts. The statement was reviewed by two external reviewers nominated by EFSA and was adopted by the CVMP on 11 April 2013.

Assessment

1. Introduction

The present statement, jointly prepared by the European Food Safety Authority (EFSA) and the European Medicines Agency (EMA), deals with the risk assessment on the presence of residues of phenylbutazone in horse meat in relation to the presence of residues of phenylbutazone in horse meat reported by several Member States, indicating an illegal use of carcasses of horses treated with this pharmacological substance.

Phenylbutazone (4-butyl-1,2-diphenyl-3,5-pyrazolidinedione) is a synthetic pyrazolone derivative with anti-inflammatory, antipyretic and analgesic properties (non-steroidal anti-inflammatory drug (NSAID)).

In veterinary medicine, phenylbutazone is not authorised for use in food-producing animals in the European Union (EU) following the evaluation by the Committee for Medicinal Products for Veterinary Use (CVMP) in 1997 (see Appendix A CVMP Summary Report). However, phenylbutazone is used to treat musculoskeletal disorders, such as rheumatoid and arthritic diseases and for pain relief in non-food producing animals including horses not destined to enter the food chain.

The presence of residues of phenylbutazone in horses being sent for slaughter indicates the presence of illegal practices, possibly occurring to facilitate the disposal of animals not intended to enter the food chain. In the EU, horses are subjected to a specific traceability system and since 2000, they are identified by a life-time identification document, named passport in accordance with Commission Regulation (EC) No 504/2008². Horse passport is a major element of the Food Chain Information (FCI) and the presence of illicit practices possibly including falsification of passports may then result in animals which have been treated with drugs not approved for food-producing animals, such as phenylbutazone, entering the food chain.

The main strengths and weaknesses of the current horse identification and FCI systems will be addressed in further detail in the EFSA Scientific Opinion on public health hazards to be covered by inspection of meat (solipeds) scheduled to be published by June 2013.

In human medicine, phenylbutazone is authorised for use in the treatment of severe cases of chronic inflammatory diseases (e.g. rheumatoid arthritis, arthritis urica, spondylitis) where a satisfactory effect cannot be achieved with other NSAIDs. The therapeutic doses typically range from 200 to 600 mg/person per day. Phenylbutazone is approved in some European Union (EU) countries only as a second line treatment with restricted use, due to its potential serious side effects.

2. Hazard identification and characterisation

Phenylbutazone is a NSAID, classified as an enolic acid, which exerts its pharmacological and toxicological effects through inhibition of the prostaglandin endoperoxide synthetase system. The substance has been evaluated by the CVMP in 1997 with regard to safety of residues to the consumer.

Following a request from the European Commission dated 28 February 2013 for an evaluation of risks to the consumer related to the presence of phenylbutazone residues in horse meat and in meat products contaminated with horse meat, experts from EMA and EFSA reviewed the main conclusions of the CVMP from 1997 regarding safety in the light of any new information that has become available.

² Commission Regulation (EC) No 504/2008 of 6 June 2008 implementing Council Directives 90/426/EEC and 90/427/EEC as regards methods for the identification of equidae. OJ L 149, 7.6.2008, p. 3-32.

EFSA and EMA based their evaluation related to the safety of phenylbutazone on previous evaluations and additional literature that became available since then.

2.1. Safety assessment

The EU requires by law that foodstuffs, such as meat, milk or eggs, obtained from animals treated with veterinary medicines used in animal husbandry must not contain any residue that might represent a hazard to the health of the consumer. Before a veterinary medicine intended for use in food-producing animals can be authorised in the EU, the safety of its pharmacologically active substances and their residues must be evaluated. This assessment is carried out by the CVMP and it is based on identifying an acceptable daily intake (ADI) of residues which is calculated from the concentration level at which no toxic effects were observed, the no observed effect level (NOEL), and applying relevant uncertainty factors.

In 1997, the CVMP has assessed the consumer safety of phenylbutazone and concluded that no ADI could be established (See Annex A CVMP Summary Report). The main areas of concern identified in relation to the substance were the lack of a NOEL for blood dyscrasias in humans, inadequate *in vivo* studies on mutagenicity while some evidence of genotoxic potential had been shown *in vitro*, no NOEL for neoplastic effects in mice and rats and inadequate data on reproductive toxicity. As a consequence it was not possible to set maximum residue limits (MRLs) for phenylbutazone and thus its use in food producing species is not permitted.

The CVMP (1997) noted that phenylbutazone is metabolised to oxyphenbutazone and γ -hydroxyphenylbutazones possibly representing the major metabolic pathways in most species. The CVMP also noted that it was unclear whether phenylbutazone or its metabolite oxyphenbutazone was the more potent substance in terms of pharmacological effects and toxicity to laboratory animals due to the limited data available.

Very limited information has become available since the evaluation carried out by the CVMP in 1997.

Limited additional information on pharmacokinetics data in horses was considered which indicates that phenylbutazone follows zero-order kinetics where longer elimination half-lives are observed as the doses increase. Administration of phenylbutazone in a horse every 24 hours for 5 consecutive days also indicated an increase of oxyphenbutazone during the 5-day dosing period.

2.1.1. Blood dyscrasias in humans

The 1997 CVMP summary report noted that the most critical known effects in humans treated with phenylbutazone are blood dyscrasias, including agranulocytosis and aplastic anaemia. The myelotoxic effects of phenylbutazone (including its metabolites) are analogous to chloramphenicol and cytotoxic drugs. Mortality from fatal bone marrow depression due to phenylbutazone and oxyphenbutazone is estimated at 2.2 and 3.8 per 100,000, respectively, compared to 2.5-5 per 100,000 for chloramphenicol. The CVMP concluded that the clinical profile of phenylbutazone-induced dyscrasias is complicated as no mechanism for phenylbutazone-induced myelotoxicity in humans has been demonstrated. Moreover, it was considered difficult to relate the findings of the *in vitro* studies to the events observed in treated human patients and no animal model exists for the myelotoxic effects. No NOEL could be determined for this effect.

Aplastic anaemia is a rare (approximately 1:30.000 cases), but life-threatening condition and has been reported in human patients following therapeutic use of phenylbutazone (therapeutic doses range typically from 200 to 600 mg/person per day). At population level, a study published in JAMA (1986) concluded that significant excess risk estimates were found for phenylbutazone and oxyphenbutazone (6.6 cases per million) when used any time in a 5-month period before hospital admission. There was

also a suggestion, with limited numbers, that the risk was higher if either phenylbutazone or oxyphenbutazone was taken regularly and for a sustained period. The risk to agranulocytosis was also increased (0.2 cases per million) in relation to exposure 7 days prior to the event.

No new relevant data became available since the CVMP evaluation in 1997 and the mechanism for the apparent myelotoxicity of phenylbutazone in humans is still unknown.

It is therefore concluded that given that a threshold level cannot be identified for the idiosyncratic reactions observed in humans, it cannot be excluded that a single exposure to therapeutic doses of phenylbutazone may cause aplastic anaemia.

2.1.2. Mutagenicity and genotoxicity

In the 1997 CVMP report several *in vitro* studies were assessed. Tests performed for gene mutation in *Salmonella typhimurium* strains resulted in negative outcomes. Negative findings were also found in silkworms but positive responses were observed in mouse cells. Several chromosomal aberration studies using hamster cells showed a significant increase in aberrations but only after metabolic activation, and negative results were observed in human fibroblasts. However, no adequate *in vivo* studies were available and therefore no conclusion could be made on the genotoxicity of phenylbutazone.

In other publicly available data, it was shown that phenylbutazone was negative for chromosomal aberrations in *in vivo* bone marrow studies in rats and Chinese hamsters. However, micronuclei results in bone marrow cells of mice were reported to be positive as well as negative.

Additional new published data also show inconsistent findings with one paper reporting that phenylbutazone was negative in bacterial mutation assays with *S. typhimurium* strains TA97a, TA98, TA100 and TA102, but caused an increase in sister chromatid exchange (SCE) in bone marrow cells of male mice, after intraperitoneal administration (50, 100 and 200 mg/kg body weight (b.w.)).

In summary the results of the tests available are inconsistent with regard to phenylbutazone's possible chromosomal effects in experimental animal studies but the weight of evidence from the *in vitro* data indicates that phenylbutazone is unlikely to cause gene mutations.

There is conflicting evidence in the human chromosomal aberration studies especially with regard to dose-response.

The review of the previous evaluation confirms that on the basis of the data available on genotoxicity it is not possible to conclude on the genotoxic potential of phenylbutazone.

2.1.3. Carcinogenicity

The 1997 CVMP summary report evaluated/considered two studies in rats and one in mice. Following a 2-year exposure to phenylbutazone, positive dose-dependent trends were observed in female rats for leukaemia, hepatic neoplasms and adrenal pheochromocytomas. In a 103-week study in rats exposed to phenylbutazone, small numbers of renal tubular cell adenomas and carcinomas were seen in male rats and tubular cell adenomas and transitional cell carcinomas of the pelvic epithelium were seen in females. Adrenal gland medullary hyperplasia was significantly increased in the high dose females. In addition, an increased incidence of histiocytic infiltration of the lung was seen in the high dose females and a statistically decreased incidence of mammary fibroadenomas was seen in the high dose females with a significant negative trend.

In a 103-week study in mice, hepatocellular adenomas or hepatocellular adenomas and carcinomas combined showed significant positive trends in male mice, whereas a significant decrease in lymphomas

was seen in high dose male mice compared to controls. Severe liver toxicity was observed in mice (see Section 2.1.5).

No NOELs for neoplastic or non-neoplastic effects could be identified from these studies.

The CVMP concluded that phenylbutazone is probably carcinogenic to male mice and female rats as indicated by the results available from these studies. No further data have been published since the CVMP report.

It was furthermore noted that the rat kidney tumours observed were associated with inflammation, papillary necrosis and mineralization, whereas the mouse liver tumours were associated with haemorrhage, centrilobular cytomegaly and kariomegaly, fatty metamorphosis, cellular degeneration and coagulative necrosis. Species/sex specificity was also observed. In both cases it would be plausible to assume that tumour formation occurred as a consequence of tissue damage/toxicity above a certain threshold. However, since genotoxicity data are inconclusive, a genotoxic mode of action cannot be excluded for rodent tumour formation.

2.1.4. Reproductive toxicity

The 1997 CVMP summary report indicated that there were no adequate reproductive or teratology studies available. However, there were two studies in rats that compared the reproductive toxicity of a number of NSAIDs and other pharmaceuticals, including phenylbutazone. Severe maternal toxicity was reported and there was evidence of fetotoxicity in offspring of female rats exposed to phenylbutazone for 14 days prior to mating through to weaning. Neonatal viability and survival were decreased in the absence of maternal toxicity as well as reductions in implantations and decreased foetal and litter weights. No NOELs could be derived from the available data.

The original CVMP conclusions on reproductive toxicity remain as no further data have become available since the CVMP report.

2.1.5. Subchronic and chronic toxicity

In the 1997 CVMP summary report several toxicity studies were described in mice, rats and cats

In a 13-week study in mice, half of the animals died. Liver weights were significantly increased at 300 or 600 mg phenylbutazone/kg b.w. per day. The NOEL for this study was 150 mg/kg b.w. per day. In a 2-year carcinogenicity study, also degeneration, haemorrhages and necrosis were observed in the liver of male mice even at the lowest dose of 150 mg/kg b.w. per day at which there was no statistically significant increase in liver tumours.

In a 13-week study in rats, similar mortality occurred as well as clinical effects including diarrhoea, and poor grooming. Body weights were found significantly lower and liver weights were significantly increased. Renal papillary necrosis, papillary oedema and mineralisation were seen at increased incidences as well as testis degeneration. Lymphoid depletion of thymus, spleen and lymph nodes was also observed. The NOEL for this study was 25 mg/kg b.w. per day.

Following an oral exposure of cats (5 animals) to phenylbutazone for 21 days, weight loss and inappetance with clinical symptoms of alopecia, dehydration, vomiting and depression were observed. Deaths occurred from day 12, with one animal surviving to the end of the study in a moribund condition. Erythrocyte count and haemoglobin concentration were decreased. Renal damage, bone marrow depression and a reduction in erythroblasts were reported at post mortem, but agranulocytosis was not detected. No toxic effects were reported in a cat that received fortnightly doses of 2 x 12-16 mg phenylbutazone/kg b.w. for one year.

In conclusion, kidney and liver toxicity were considered the most relevant toxic effects identified in rats and mice, respectively. While no NOEL could be established for hepatotoxicity from the chronic toxicity studies, the lowest NOEL identified for rats for nephrotoxicity in a sub-chronic study was 25 mg/kg b.w. per day.

2.2. Conclusions on hazard identification and characterisation

Since the CVMP assessment in 1997, very limited new information on phenylbutazone has emerged in the literature.

The conclusions on the hazard identification and characterisation can be summarised as follows:

- The most critical potential safety findings related to toxicity of phenylbutazone were the idiosyncratic effects on bone marrow (blood dyscrasias including aplastic anaemia). While aplastic anaemia is a rare event after therapeutic use in human ($\approx 1:30.000$) it is nevertheless a potential life-threatening condition. The mechanism is unknown and it is not possible to determine a threshold for blood dyscrasias.
- For mutagenicity the results indicate no genotoxic potential on bacterial systems, and equivocal results in mammalian systems. Although at present the weight of evidence indicates that phenylbutazone is unlikely to show relevant genotoxic effects under *in vivo* conditions, no conclusive *in vivo* genotoxicity studies are available and hence no final conclusion can be drawn.
- Phenylbutazone is probably carcinogenic to male mice (liver tumours) and female rats (kidney/urinary tract tumours) as was concluded in the 1997 CVMP report. The hepatotoxicity and nephrotoxicity seen, including significant pathological effects, indicate a potential for a (non-geno)toxic nature of tumour induction; however, the genotoxicity data are not conclusive and therefore no firm conclusion can be made on the carcinogenic mode of action. No NOELs following chronic exposure for neoplastic or non-neoplastic effects could be identified.

- No adequate data on reproductive toxicity are available and therefore no NOELs can be derived.
- Nephrotoxicity and hepatotoxicity are considered to be the main toxic effects in rats and mice, respectively. The lowest NOEL of 25 mg/kg b.w. was identified for nephrotoxicity from a sub-chronic study in rats.

3. Exposure assessment

3.1. Prevalence of phenylbutazone in horse samples

The occurrence data considered in this scientific statement were collected in the framework of the National Residue Control Plans (NRCP), for which the results are published in annual reports. However, these reports could not be used because inherent limitations already underlined by EFSA (EFSA, 2010) and specific data requests had to be sent to the individual Member State.

The NRCP results from 19 Member States collated in the framework of this scientific statement, representing a total of 2,386 samples taken from years 2005 to 2013 were used (Table B1, Appendix B).

A limitation of this dataset is represented by the high heterogeneity present in the analysed matrices, the sampling strategies and analytical performances. In particular it is noted that the presence of phenylbutazone is monitored in different matrices including kidney, liver, serum, plasma and muscle. Substantial variability of the sample proportion was observed among different Member States with in some cases an extremely low number of samples. Finally, some differences of around a factor of 10 were observed in the limits of quantification or CC_{alpha} reported for the different matrices.

Overall, 37 samples (1.6 %) were reported for phenylbutazone. Kidney was the matrix with the highest detection rate (2.8 % positive samples corresponding to 33 out of 1160 kidney samples) and highest levels measured (from traces up to 1,900 µg/kg, with a median at 4.0 µg/kg). Only one sample of muscle out of 672 (0.1 %) was reported positive, with phenylbutazone at 19.2 µg/kg.

From these data, the prevalence of horse carcasses testing positive for phenylbutazone was calculated to be on average 0.13 %, weighted according to annual production across EU countries. A statistical analysis performed in order to assess the uncertainty around the prevalence of horse carcasses containing phenylbutazone in the EU showed that, due to the low number of samples available in some countries, the prevalence at the EU level would be below 7.2 % with a 95 % confidence level (see Appendix D). The UK is among the Member States which reported the highest detection rates of phenylbutazone in horse samples in the last years, with a 3.5 % percentage of positive samples (17/480) since the beginning of 2013 in the framework of the UK-Food Standards Agency (FSA) '100 % testing of horse carcasses'. This prevalence of 3.5 % was retained as a high estimate for the EU prevalence. This estimate was within the uncertainty range of the statistical analysis performed.

3.2. Prevalence of beef-based products adulterated with horse meat

The presence of horse meat³ in beef-based products was estimated considering the results from industry tests reported by the UK-Food Standards Agency (FSA) and the Food Safety Authority of Ireland (FSAI), from the UK wide survey of beef products and from the RASFF notifications. Overall, horse meat was found in around 1 % of the samples tested, with 2 % being considered a high bounding estimate. The beef-based products most frequently found positive for horse meat were pasta with meat,

³ In the context of adulterated beef based products, the term horse meat used in this statement refers indifferently to presence of equine DNA above 1 %, or to equine proteins.

meat burgers, meat balls, beef goulash, beef stewed and frozen pieces of beef. The percentage of horse meat measured represented up to 100 % of the meat content in pasta with meat, 90 % of the meat content in burgers and meatballs, 80 % of the meat stewed, and 70 % of the meat content in the other beef based products (see Appendix B for further details).

3.3. Likelihood of dietary exposure to phenylbutazone

The likelihood of dietary exposure to phenylbutazone was estimated at the consumer level by combining the frequency of consumption of horse meat or beef-based products potentially adulterated with horse meat (details on consumption data reported in Appendix B) with the prevalence of phenylbutazone in horse meat. An estimate of the number of individuals per 100 million potentially exposed each day to phenylbutazone was then derived at the population level by combining the likelihood of exposure at the individual level together with the estimated number of consumers of horse meat or beef-based products within the population.

The estimated likelihood of dietary exposure to phenylbutazone is higher for the consumers of horse meat than for the consumers of beef-based products potentially adulterated with horse meat. Based on a horse meat consumption frequency of twice a week and a prevalence of phenylbutazone in horse meat at 3.5 %, a consumer would be exposed to phenylbutazone once every 4 months. Using a conservative scenario (beef-based products consumption frequency of four times a week, 2 % of the beef-based products containing horse meat, 3.5 % (see Section 3.1) of horse meat containing phenylbutazone), a consumer of beef-based products could be exposed to phenylbutazone once every 6.8 years.

At the population level, and across different country and age groups this would represent from up to 144 to up to approximately 30,300 individuals per 100 million potentially exposed each day to phenylbutazone through the consumption of horse meat or horse meat products, and from up to 5 to up to 36,800 individuals per 100 million through the consumption of beef-based products adulterated with horse meat. The details on the exposure likelihood estimations are reported in Appendix B.

3.4. Levels of exposure to phenylbutazone

Considering the likelihood of exposure to phenylbutazone, only infrequent events of exposure are foreseen at the individual level for both horse meat consumers and for consumers of beef-based products potentially adulterated with horse meat. For this reason, the estimation of chronic exposure levels were not considered relevant, and only the acute levels resulting from a single day of exposure were estimated. The acute levels of exposure were higher in children than in adolescents and in adults.

Assuming exposure via horse meat contaminated at the highest levels measured in horse samples (1,900 µg/kg phenylbutazone measured in kidney), the average acute exposure from the consumption of horse meat or adulterated beef-based products was estimated to range across the population groups from 2.24 to 9.69 µg/kg b.w. per day for children and from 1.38 to 4.42 µg/kg b.w. per day for adolescents and adults. The 95th percentile acute exposure was estimated to range across the population groups from 4.50 to 15.55 µg/kg b.w. per day for children, and from 2.74 to 9.00 µg/kg b.w. per day in adolescents and adults. When considering horse meat containing phenylbutazone at a level equal to that measured in the only positive muscle sample reported (19.2 µg/kg), average acute

exposure levels ranging from 0.02 to 0.10 µg/kg b.w. per day for the children and from 0.01 to 0.04 µg/kg b.w. per day for the adolescents and adults were estimated. The 95th percentage acute exposure levels were estimated to range from 0.05 to 0.16 µg/kg b.w. per day for the children, and from 0.03 to 0.09 µg/kg b.w. per day for the adolescents and adults. The details on the exposure level estimations are reported in Appendix B.

4. Risk characterisation

The main hazards identified for residues of phenylbutazone are blood dyscrasias, genotoxicity and carcinogenicity.

Blood dyscrasias

The occurrence of blood dyscrasias following therapeutic use of phenylbutazone (with therapeutic doses ranging from 200 to 600 mg/person per day) in humans is low (approximately 1 in 30,000). However it is considered that this effect might occur at lower exposure levels in sensitive humans.

Given that a threshold level cannot be set for the idiosyncratic reactions observed in humans, a risk to human health following exposure to any amount of phenylbutazone, although low, cannot be excluded.

In the absence of a threshold for the idiosyncratic effects of phenylbutazone, no quantitative risk characterisation can be carried out. However the likelihood that cases of blood dyscrasias could arise from dietary exposure to phenylbutazone as a result of the recent fraudulent practices, was estimated by considering the overall likelihood that susceptible subjects were exposed to the substance (either via direct consumption or contaminated horse meat or the consumption of beef products containing contaminated horse meat). Considering the different scenarios, the daily probability for an individual both to be a subject susceptible to develop anaemia and to be exposed to phenylbutazone was estimated to range from 2 in a trillion up to 1 in 100 million.

Genotoxicity/carcinogenicity

Data on genotoxicity are equivocal, and it is not possible to conclude that a genotoxic mode of action is responsible for the carcinogenicity observed in laboratory animals. However it is noted from studies in rodents that the tumours are most associated with organs that also show adverse effects at the lowest doses tested, indicating that the carcinogenicity might be linked to organ toxicity rather than direct genotoxicity. The doses at which organ toxicity and carcinogenicity were observed are more than three orders of magnitude higher than those that could be expected from potential exposure to phenylbutazone from horse meat. In addition those effects were observed following chronic exposure which is unlikely to be the case in relation to the frequency of exposure events to humans from the consumption of horse meat containing phenylbutazone. Therefore it is considered that the risk of carcinogenicity to humans from this exposure is of very low concern.

5. Uncertainty analysis

An evaluation of the inherent uncertainties in the assessment of exposure to phenylbutazone has been performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006). In addition, the report on 'Characterizing and Communicating Uncertainty in Exposure Assessment' has been considered (WHO-IPCS, 2008).

The summary of uncertainties is reported in Table 1. A full discussion of the uncertainties identified is given in Appendix D.

The uncertainties are mainly related to the limited information available to estimate the prevalence and levels of phenylbutazone in horse meat, the prevalence of fraudulent practices which entails replacing beef meat by horse meat in beef-based products, and the consumption habits of horse meat throughout Europe. However, assumptions were made in this regard in order to be conservative.

Table 1: Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of the exposure of phenylbutazone in horse meat.

Sources of uncertainty	Direction ^(a)
Occurrence data originating from target sampling, which may not be representative	+
Low number of samples available in some countries in regards to their annual production level of horse carcasses	+/-
Occurrence and maximum levels of phenylbutazone measured in parts of the animals not intended for consumption were taken as representative of the levels in horse meat.	+
Uncertainty related to the assumption that 90 % of processed beef meat was replaced by horse meat in meat burgers/meatballs, 80 % in beef stewed and 30 % in minced/grounded beef	+
Uncertainty related to the assumption that 0.1, 1 and 2 % of the beef-based products would contain horse meat	+
Uncertainty related to the assumption that horse offal could be illicitly used in beef-based products	+
Insufficient data on the presence of oxyphenbutazone in horse meat	-
Limited reliability of the data on horse meat consumption available in the EFSA Comprehensive European Food Consumption Database.	+/-
Consumption surveys not detailing the kind of meat used in meat products and reporting data disaggregated at the ingredient level for processed beef meat products	+/-
A single estimate of prevalence at European level without considering the possible variability throughout Europe	+/-
Incidence of aplastic anaemia observed in the therapeutic use of the substance considered to calculate the probability of potential cases of blood dyscrasias in relation to exposure from horse meat containing phenylbutazone	+

(a): + = uncertainty with potential to cause over-estimation of exposure/risk; - = uncertainty with potential to cause under-estimation of exposure/risk

It is important to acknowledge that the current assessment is restricted to horse meat and to potentially adulterated beef-based products. The presence of residues of phenylbutazone has been rarely reported in bovine animals (EFSA, 2011a, 2012, 2013), which has not been specifically taken into account in the estimation of the likelihood of exposure. Moreover, due to the lack of occurrence data on oxyphenbutazone and on its concurrent presence with phenylbutazone, this metabolite has not been taken into consideration in the exposure assessment.

It is considered that the impact of the uncertainties on the risk assessment regarding human exposure to residues of phenylbutazone in horse meat is considerable, however, it can be concluded that the risk assessment of human exposure considered in the statement is likely to overestimate the risk.

Conclusions and recommendations

Conclusions

Phenylbutazone is not authorised for use in food-producing animals as maximum residue limits are not established for the substance. In 1997, the Committee for Medicinal Products for Veterinary Use (CVMP) assessed the consumer safety of phenylbutazone and concluded that no acceptable daily intake (ADI) could be established hence no maximum residue limits (MRL) could be recommended. Controls in Member States have however revealed the presence of residues of phenylbutazone in horse meat which indicate an illegal use of carcasses of horses treated with this pharmacological substance.

On 28 February 2013, the European Commission asked the European Food Safety Authority and the European Medicines Agency for a joint statement regarding a risk assessment on residues of phenylbutazone in horse meat, from direct consumption of horse meat as well as from other products adulterated with such food.

It must be noted that the risk assessment has been performed on the basis of limited data in terms of toxicity of phenylbutazone as well as in terms of actual occurrence data reported in horse meat across the EU, which had been sampled in the previous years only occasionally, using diverse sampling and detection methods. In view of the assumptions made to address most of the existing uncertainties, it was concluded that the current risk assessment is likely to overestimate the risks.

The European Food Safety Authority and the European Medicines Agency conclude that:

- The safety assessment performed by the CVMP regarding phenylbutazone in 1997 has been confirmed in the absence of any relevant new data. Therefore EMA and EFSA confirmed that it is not possible to establish safe levels for phenylbutazone in food. In relation to the risk to the consumer, the main concerns remain idiosyncratic blood dyscrasias and the genotoxic/carcinogenic potential. No thresholds could be identified.
- The occurrence of blood dyscrasias following therapeutic use of phenylbutazone in humans is low (approximately 1 in 30,000). In the absence of dose response data it is considered that this effect might occur at lower exposure levels in sensitive humans.
- Data on genotoxicity are equivocal and a genotoxic mode of action for carcinogenicity cannot be excluded but it is considered most likely that the carcinogenicity observed in laboratory animals might be linked to organ toxicity.
- The results from the National Residue Control Plans (NRCP) from 19 Member States collated in the framework of this scientific statement were used to estimate the prevalence of horse meat containing phenylbutazone and the level to which consumers may have been exposed.
- Thirty seven samples out of 2,386 samples taken from years 2005 to 2013 were found positive for phenylbutazone. Kidney was the matrix with the highest detection rate (2.8 % (33/1,160) and highest levels measured (up to 1,900 µg/kg). Only one sample of muscle out of 672 (0.1 %) was found positive, with phenylbutazone at 19.2 µg/kg.
- The likelihood of dietary exposure to phenylbutazone was estimated at the consumer level by combining the frequency of consumption of horse meat or beef-based products potentially adulterated with horse meat with the prevalence of phenylbutazone in horse meat.
- Based on a horse meat consumption frequency of twice a week and a prevalence of phenylbutazone in horse meat of 3.5 %, which is considered as a conservative scenario, the probability of exposure of a consumer to phenylbutazone would be once every 4 months. Lower probabilities of exposure

were estimated for consumption, from two to four times a week, of beef-based products potentially adulterated with horse meat.

- At the population level and across different countries and age groups, up to 144 individuals to up to approximately 30,300 individuals per 100 million have been potentially exposed each day to phenylbutazone through the consumption of horse meat or horse meat products, and up to 5 to up to 36,800 individuals per 100 million have been potentially exposed each day through the consumption of beef-based products adulterated with horse meat.
- Considering the infrequent events of exposure, only the acute levels resulting from a single day of exposure were estimated. The estimated acute levels of exposure were higher in children than in adolescents and in adults.
- Assuming horse meat contaminated at the highest levels measured in horse samples (measured in a kidney sample), an average acute exposure up to 9.69 µg/kg body weight (b.w.) per day, and a 95th percentile acute exposure up to 15.55 µg/kg b.w. per day were estimated from the consumption of horse meat or adulterated beef-based products. Lower exposure can be expected due to the lower concentration likely to be found in muscle in comparison to kidney.
- There is uncertainty about the risk to potential exposure to oxyphenbutazone which was not taken into account in the current assessment. Oxyphenbutazone was also detected in one muscle sample at a concentration higher than phenylbutazone in the same sample. Oxyphenbutazone is a major metabolite in horses with a slower elimination following daily administration to horses which is often the case in veterinary practice.
- In the absence of a threshold of toxicity for the idiosyncratic effects of phenylbutazone, no quantitative risk characterisation could be carried out. However, the likelihood that cases of blood dyscrasias could arise from dietary exposure to phenylbutazone as a result of the recent fraudulent practices was estimated by considering the overall likelihood that sensitive subjects were exposed to the substance (either via direct consumption or contaminated horse meat or the consumption of beef products containing contaminated horse meat). Considering the different scenarios, on one given day the probability for an individual both to be a subject susceptible to develop aplastic anaemia and to be exposed to phenylbutazone was estimated to range approximately from 2 in a trillion to up to 1 in 100 million.
- The doses at which organ toxicity was observed in experimental studies in laboratory animals are more than three orders of magnitude higher than those that could be expected from potential exposure to phenylbutazone from horse meat consumed as such or present in beef-based products. In addition those effects were observed following chronic exposure which is unlikely to be the case in relation to the frequency of exposure events from the consumption of horse meat containing phenylbutazone. Therefore it is considered that the risk of carcinogenicity to humans from this exposure is of very low concern.

Recommendations

The findings of residues of phenylbutazone in horsemeat results from horse carcasses entering the food chain illegally. With the aim of improving controls and minimising the risk the following recommendations are made:

- A reliable identification system to improve traceability of horses, including strengthening the “horse passport” system, with appropriate and effective enforcement.

- As the sampling intensity and methods for horses varies substantially between Member States, harmonised control measures in terms of sampling and performance of analytical methods in relation to phenylbutazone should be considered.
- Improvement of data reporting methodology concerning monitoring of veterinary drug residues and other substances by Member States under Council Directive 96/23, as recommended by EFSA (2012).
- Monitoring of the main metabolite oxyphenbutazone considering its slower elimination in horses and the likely similar pharmacological effects and toxicity of oxyphenbutazone and phenylbutazone.

References

- EFSA (European Food Safety Authority), 2006. Guidance of the Scientific Committee on a request from EFSA related to Uncertainties in Dietary Exposure Assessment. The EFSA Journal, 438, 1-54.
- EFSA (European Food Safety Authority), 2012. Technical report of EFSA: Evaluation of the data collection performed in the framework of Directive 96/23/EC, 20 pp. Available online: www.efsa.europa.eu
- EFSA (European Food Safety Authority), 2011a. Report for 2009 on the results from the monitoring of veterinary medicinal product residues and other substances in live animals and animal products. Supporting Publications 2011:158, 70 pp.
- EFSA (European Food Safety Authority), 2011b. Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment. EFSA Journal 2011;9(3):2097, 34 pp.
- EFSA (European Food Safety Authority), 2012. Report for 2010 on the results from the monitoring of veterinary medicinal product residues and other substances in live animals and animal products. Supporting Publications 2012:212, 65 pp.
- EFSA (European Food Safety Authority), 2013. Report for 2011 on the results from the monitoring of veterinary medicinal product residues and other substances in live animals and animal products. To be published.
- Food Safety Authority of Ireland (2013a); Results of Industry Tests for Horse Meat up to 1st March 2013. [2 pp.]. Available online: <http://www.fsai.ie>
- Food Safety Authority of Ireland (2013b); Results of Industry Tests for Horse Meat from 2nd to 25th March 2013. [1 pp.]. Available online: <http://www.fsai.ie>
- Food Safety Authority of Ireland (2013c); FSAI Advises of Findus Beef Lasagne Withdrawal in Ireland. Press release of the 8th February 2013. Available online: <http://www.fsai.ie>
- Food Safety Authority of Ireland (2013d); ALDI Frozen Beef Lasagne and Spaghetti Bolognese Test Positive For Horse Meat. Press release of the 8th February 2013. Available online: <http://www.fsai.ie>
- United Kingdom-Food Standards Agency (2013a); Third summary report on results of industry testing of meat products – 1st March 2013. [4 pp.]. Available online: <http://www.food.gov.uk>
- United Kingdom-Food Standards Agency (2013b); Update results of FSA UK-wide survey of beef products. Press release of the 26th March 2013. Available online: <http://www.food.gov.uk>
- Summary report CVMP Phenylbutazone (1997). EMEA/MRL/297/97-FINAL. October 1997
- The United States Pharmacopeial Convention on Phenylbutazone (2004)
- Soma, L.R., Gallis, D.E., Davis, W.L., Cochran T.A., Woodward C.B. (1983). Phenylbutazone kinetics and metabolite concentrations in the horse after five days of administration. Am J. Vet. Res. 1983 Nov; 44(11):2104-9.
- Risks of Agranulocytosis and Aplastic Anemia. (1986) JAMA, Oct 3, 1986-Vol 256, No. 13 (1749-1757)
- Gebhart, E., Wissmuller, H. F. (1972). Investigations on the effect of phenylbutazone on chromosomes and mitosis in the bone marrow of rats. Mutation Research, 17 (1973) 283-286
- Muller, D., Strasser, F.F. (1986). Comparative studies on the Chinese hamster bone marrow after treatment with phenylbutazone and cyclophosphamide. Mutation Res., 13 (1971) 377-382

- Pentiah, P.R., Reddy, P.P., Reddi, O.S. (1980). Induction of micronuclei in bone marrow cells of mice treated with phenylbutazone. *Indian J.Exp. Biol.* 18:869-871.
- Charles, D., Leonard A. (1978). Mutagenicity tests with phenylbutazone in mammals. *Toxicology Letters* Volume 2, Issue 4, October 1978, 225–230
- Giri, A.K., Mukhopadhyay, A. (1998). Mutagenicity assay in Salmonella and in vivo sister chromatid exchange in bone marrow cells of mice for four pyrazolone derivatives. *Mutation research* 420 (1998) 15- 25
- Kari, F., Bucher, J., Haseman, J., Eustis, S., Huff, J. (1995). Long-term exposure to the anti-inflammatory agent Phenylbutazone induces kidney tumors in rats and liver tumors in mice. *Jpn. J. Cancer Res.* 86, 252-263, March 1995
- Cast, I.P. (1961). Phenylbutazone and Leukaemia. *Brit. med.J.*, Dec 1961. 1569-1570
- Woodcliff, H.J., Dougan, L. (1964). Acute Leukaemia Associated With Phenylbutazone Treatment. *Brit. med.J.*, 1964, 1, 744-746
- Thorpe, G.J. (1964). Leukaemia and Phenylbutazone. *Brit. med.J.*, June 1964, 1707.
- NTP Technical Report on the Toxicology and Carcinogenesis Studies of Phenylbutazone (CAS No. 50-33-9) in F344/N Rats and B6C3F1 Mice. National Toxicology Program. Technical report series No. 367. U.S. Department of Health and Human Services. NIH Publication No. 90-2822. March 1990.
- Galloway, S., Lorge, E., Aardema M.J., Eastmond, D., Fellows, M., Heflich, R., Kirkland, D., Levy, D.D., Lynch, A. M., Marzin, D., Morita, T., Schuler, M., Speit G. (2011). Workshop summary: Top concentration for in vitro mammalian cell genotoxicity assays; and report from working group on toxicity measures and top concentration for in vitro cytogenetics assays (chromosome aberrations and micronucleus). *Mutation Research* 723 (2011) 77– 83
- Schardein, J.L., Blatz, A.T., Woosley, E.T., Kaump, D.H. (1969). Reproduction Studies on Sodium Meclofenamate in comparison to Aspirin and Phenylbutazone. *Toxicology and Applied Pharmacology* 15, 46-55 (1969)
- WHO-IPCS (World Health Organization – International Programme on Chemical Safety), 2008. Uncertainty and Data Quality in Exposure Assessment. Part.1 Guidance Document on Characterizing and Communicating Uncertainty in Exposure Assessment. Available online: http://www.who.int/ipcs/publications/methods/harmonization/exposure_assessment.pdf

Appendices

A. Summary report CVMP (1997)



European Medicines Agency
Veterinary Medicines and Inspections

EMEA/MRL/297/97-FINAL
October 1997

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

PHENYLBUTAZONE

SUMMARY REPORT

1. Phenylbutazone (4-butyl,1,2-diphenylpyrazolidone-3,5-dione) is a synthetic pyrazolone derivative with anti-inflammatory, antipyretic and analgesic properties. In veterinary medicine phenylbutazone is used in mono-preparations by oral or parenteral administration, or in conjunction with ramifenazone and is administered by intramuscular or slow intravenous injection. Phenylbutazone preparations are indicated for use in horses as an adjunct to therapy in many inflammatory conditions of the musculoskeletal and locomotor systems. Recommended dosages are between 2.2-9 mg/kg bw/day for a maximum of 12 days. A 2:1 combination product of ramifenazone and phenylbutazone is available for similar indications in horses, cattle and pigs. The recommended dose is 7 mg/kg bw for horses and cattle parenterally, 14 mg/kg bw for calves and pigs parenterally, or 20 g/horse or 8 g/pig rectally.
2. Phenylbutazone is a non-steroidal anti-inflammatory drug (NSAID), classified as an enolic acid. In common with other non-steroidal anti-inflammatory drugs phenylbutazone exerts its pharmacological and toxicological effects through inhibition of the prostaglandin endoperoxide synthetase system. The ED₅₀ for the inhibition of prostaglandin synthesis in a carrageenin exudate study in the rat was 3 x 2.8 mg phenylbutazone/kg bw/day. Phenylbutazone has variable antipyretic activity in different species. In the rat the effective dose is approximately 140 mg/kg. In the rabbit, pyrexia was reduced by 70-80%, the effect lasting for at least 3 hours after intravenous doses of 40 or 70 mg/kg phenylbutazone. Oral administration of doses of phenylbutazone of 20-200 mg/kg to mice, increased reaction time to thermal irritation and hot plate exposure by around 20-30% and 30-40%, respectively in a dose-dependent manner. Intraperitoneal administration of phenylbutazone in mice inhibits acetic acid-induced abdominal pain. The ED₅₀ was reported as 20 mg/kg. In the cat, intravenous doses of phenylbutazone have a normotensive or hypertensive effect on blood pressure; phenylbutazone has no sedating or spasmolytic effects. Some non-steroidal anti-inflammatory drugs such as phenylbutazone and aspirin irreversibly inhibit platelet cyclo-oxygenase, blocking formation of thromboxane A₂, thus inhibiting platelet aggregation, thrombosis and haemostasis. It was not possible to determine a pharmacological NOEL from the data available.
3. Radiolabel studies in the rat indicated rapid and almost complete absorption of phenylbutazone following oral exposure. In male rats dosed with 15 mg/kg bw, over 50% of the dose was excreted in the urine within 24 hours. After 48 hours approximately 95% of the total dose was recovered, 60% in urine and 35% in faeces. In female rats given doses of 10 mg/kg bw by gavage, about 88% of the dose was recovered in the urine and faeces within 96 hours. Phenylbutazone binds strongly to plasma proteins with around 1/4-1/3 of the dose localised in the plasma and binding in plasma of over 90% being reported in various species. In rats and dogs the highest tissue concentrations are seen in the kidney and liver.

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 84 47

E-mail: mail@emea.europa.eu <http://www.emea.europa.eu>

© European Medicines Agency, 2008. Reproduction is authorised provided the source is acknowledged.

4. A number of metabolites have been identified, including oxyphenbutazone, γ -hydroxyphenyl-butazone, p- γ -hydroxyphenylbutazone, and C-glucuronides of phenylbutazone and γ -hydroxy-phenylbutazone. Metabolism to oxyphenbutazone and γ -hydroxyphenylbutazones appears to represent the major metabolic pathway in most species. Metabolism and excretion of phenylbutazone were measured in the rat following administration of a radiolabelled dose of 15 mg/kg bw by oral gavage. Comparison of faecal and biliary excretion suggested that entero-hepatic recirculation was insignificant. The elimination half-life in most laboratory animals, pigs and horses is around 5-8 hours, except for goats (15 hours) and cattle (37-66 hours).
5. In humans rapid and almost complete absorption from oral administration (71-89% excretion) is reported. Intramuscular absorption is relatively slow. Following administration of 800 mg doses to humans by oral or intramuscular routes absorption was greater by the oral route, plasma levels peaking at two hours. Intramuscular absorption peaked between 6-10 hours, by 24 hours plasma levels were similar for both routes. It is reported that phenylbutazone is extensively metabolised in humans with about only 1% being excreted unchanged in the urine. About 50% of the excreted dose is composed of C-4 glucuronides of phenylbutazone (40%) and γ -hydroxyphenylbutazone (12%) and only about 10% other known metabolites. In humans highest tissue levels were reported in post-mortem samples from adrenals, kidney and lung. Excretion in humans is slow, 21 days after application of a single 400 mg labelled dose only 88% (61% in urine and 26% in faeces) of the radioactivity had been recovered. The elimination half-life in humans is 66-88 hours. The major differences in metabolism between humans and laboratory species appear to be that in humans both C-glucuronidation and hydroxylation are involved, whereas in the rat and dog metabolism is predominantly through hydroxylation. It is claimed that this may account for the difference in elimination rates. In humans half-life is dose-dependent, increasing with dose over the therapeutic dose-range (1.7-3 mg/kg bw).
6. The study of phenylbutazone half-life in monozygotic and dizygotic twins showed little difference in half-life between monozygotic twins, but significantly greater differences between dizygotic twins. This indicates that a genetic element may be responsible for the wide variation in phenylbutazone metabolism in humans. Pretreatment with oxyphenbutazone increases the half-life in the rat and decreases plasma clearance. Phenobarbitone decreases the half-life of phenylbutazone. This can result in increased plasma levels of unbound pharmacologically active parent compound. Phenylbutazone is known to interact with the pharmacokinetics of many other compounds, including hydrocortisone, tolbutamide and aminopyrine, cholestyramine, anticoagulants, sulphonylureas, phenytoin, methotrexate and lithium, leading to increased toxicity.
7. Acute oral LD₅₀ values are reported for the mouse (442-700 mg/kg bw), rat (360-1000 mg/kg bw) and guinea pig (250-1220 mg/kg bw). Necrotic ulcers and gastric haemorrhage were reported in rats dosed orally with 300 mg/kg phenylbutazone and sacrificed between 4-24 hours after dosing. Significant diurnal effects were reported in male rats; oral LD₅₀ value were 710 mg/kg bw a.m., and 525 mg/kg bw p.m. In a single dose study comparing the ulcerogenic effects of 10 non-steroidal anti-inflammatory drugs, phenylbutazone showed one of the lowest ulcerogenic indices.
8. In 19-day repeated dose studies groups of 5 male and 5 female mice received daily oral doses of 0, 40, 80, 150, 300 or 600 mg/kg bw by gavage. No effects on body weights or clinical signs were reported. In rats (5 males and 5 females/group) receiving oral daily doses of 0, 40, 80, 150, 300 or 600 mg/kg bw for 19 days, 3 males and 4 high-dose females and 2 females at 300 mg/kg died due to phenylbutazone treatment, all deaths occurring on days 2-20 of treatment. Body weights were significantly depressed at doses greater than 300 mg/kg bw. NOELs could not be determined from these studies as no data on haematology, clinical chemistry or histopathology were reported.

9. In a 13-week study, groups of 10 male and 10 female mice received daily oral doses (5 days/week) of 0, 40, 80, 150, 300 or 600 mg phenylbutazone/kg bw. Five male and 4 female high dose animals died before the end of the study, but no effects on bodyweights or clinical signs were observed. Liver weights were significantly increased in both sexes at 300 and 600 mg/kg bw. No treatment-related pathology was observed. The NOEL was 150 mg/kg bw. Groups of 10 male and 10 female rats received daily oral doses (5 days/week) of 0, 25, 50, 100, 200 or 300 mg/kg bw. Seven male and 8 female high dose animals, and male and 2 females at 200 mg/kg bw died between weeks 1-9 of the study. Clinical effects reported at 200 and 300 mg/kg bw included diarrhoea, poor grooming and chromodachryorrhea. Bodyweights were significantly lower in the 200 and 300 mg/kg bw groups and liver weights were significantly increased. Renal papillary necrosis, papillary oedema and mineralisation were seen at increased incidences at doses greater than 100 mg/kg bw in males and greater than 50 mg/kg bw in females. Testicular degeneration was seen in 4/6 males at 300 mg/kg, 2/10 males at 200 mg/kg and 1/10 males at 100 mg/kg. Lymphoid depletion of thymus, spleen and lymph nodes was seen in 6/7 males and 6/8 females at 300 mg/kg and 1 male and 1 female at 200 mg/kg, this lesion was only seen in animals dying before the end of the study. The NOEL was 25 mg/kg bw.
10. In a chronic study, single dogs were given 10, 100 or 200-250 mg phenylbutazone/kg bw/day orally for 90 days. No adverse effects were seen in the animals receiving 10 or 100 mg/kg. The high dose animal developed vomiting, bloody diarrhoea and soft stools after 2 days. Soft stools were seen in the second week, inappetance and anorexia developed at week nine until death in week 12. Haematology was normal in the lower dose animals. The high dose animal was mildly anaemic throughout. No treatment related lesions were found grossly or at microscopic examination of liver spleen kidney or bone marrow. This study was not conducted to GLP standards and a NOEL could not be determined due to the small number of animals used and the limited laboratory investigations conducted.
11. Cats were exposed to oral daily doses of 44 mg phenylbutazone/kg bw for 21 days. Weight loss and inappetance occurred with clinical symptoms of alopecia, dehydration, vomiting and depression. Deaths occurred from day 12, one animal survived to the end of the study in a moribund condition. Erythrocyte count and haemoglobin concentration were decreased. Renal damage, bone marrow depression and a reduction in erythroblasts were reported at post mortem, but agranulocytosis was not detected. No toxic effects were reported in a cat that received fortnightly doses of 2 x 12-16 mg phenylbutazone/kg bw for one year.
12. Adverse clinical signs of intolerance reported in horses include depression, listlessness, inappetance, gingival and lingual ulceration, diarrhoea, anorexia, abdominal and preputial oedema, recumbency, shock and death. Necropsy findings include ulceration of the oral mucosa, stomach and (unlike other species) large bowel, with peritonitis. Renal papillary necrosis has been observed in horses but it is suggested that this may not be a direct effect of phenylbutazone, but due to dehydration or coadministration of other nephrotoxic drugs, however, use is contraindicated in these circumstances. Hepatotoxicity has been reported in horses given oral doses of 8.8 mg/kg bw for four days or 7.5 mg/kg bw for eight days. The therapeutic window is reported to be quite narrow and adverse effects have been reported at doses close recommended dose rates (single doses of 8-12 mg/kg bw for more than four days or twice daily doses of 4.4 mg/kg bw for four days, then 2.2 mg/kg bw for seven days).

13. No adequate reproductive or teratology studies were available. Two studies compared the reproductive toxicology of a number of NSAIDs and other pharmaceuticals, including phenylbutazone. There was evidence of foetotoxicity in offspring of female rats exposed to a dietary dose of 42 mg phenylbutazone/kg bw/day for 14 days prior to mating through to weaning. Severe maternal toxicity was also reported. Neonatal viability and survival were decreased, in the absence of maternal toxicity, in offspring of female rats exposed to diets containing 44 mg/kg bw/day from day 15 of gestation through weaning. Doses of 50, 100 or 200 mg phenylbutazone/kg bw by oral gavage to pregnant rats from days 6 to 15 of gestation, resulted in reductions in implantations and decreased foetal and litter weights. Investigations of teratogenicity in the rat and rabbit were inconclusive. No NOELs could be derived from these studies.
14. An *in vitro* test for gene mutation using *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and doses of phenylbutazone of 33-10000 µg/plate, with and without metabolic activation (rat and hamster S9) was negative. Doses greater than or equal to 3333 µg phenylbutazone/plate were toxic to strain TA100 in the absence of metabolic activation. An *in vitro* study for gene mutation in mammalian cells using the mouse L5178Y/TK lymphoma assay produced significant positive responses at doses greater than or equal to 500 µg/ml without metabolic activation, and greater than or equal to 400 µg/ml with metabolic activation. Lethality was reported at doses of 600 and 800 µg/ml with and without metabolic activation. Negative findings were reported in further Ames *Salmonella* assays, *B. subtilis* rec⁻/rec⁺ assays and mutagenicity studies in silkworms.
15. *In vitro* assays for chromosomal aberrations and sister chromatid exchanges (SCE) were conducted using Chinese hamster ovary (CHO) cells. No increases in SCEs were found either with or without metabolic activation. No increase in chromosome aberrations were seen without metabolic activation, significantly increased incidence of aberrations were seen with metabolic activation at 1600 and 5000 µg/ml, but not 500 µg/ml. Positive results for chromosomal aberrations have also been reported in cultured hamster lung fibroblasts (without activation), but negative results were obtained for chromosomal aberrations or SCEs in human fibroblasts. An *in vitro* study exposing human blood cells to phenylbutazone at levels of 100 mg % (cf steady-state plasma concentration at 300 mg/day of 60-130 mg %) for 16 or 40 hours, found no evidence of chromosomal or chromatid damage.
16. Chromosomal changes were seen in lymphocytes of humans exposed to prolonged administration of phenylbutazone. The frequency of cells with chromosomal damage was significantly higher in patients taking daily doses of phenylbutazone of 100-500 mg (2%) than controls (0.48%), as was the frequency of dicentric chromosomes (0.45% cf 0.06%). There was no evidence of any relationship between frequency of damaged cells and sex, age, duration of treatment or accumulated dose.
17. Patients presenting at clinic with indications for phenylbutazone therapy were tested before and during treatment. Only 3/9 patients had cells with chromosome damage (3/900 cells examined), prior to treatment. During treatment, chromosomal damage was found in 8/9 nine (62/3,000 cells). Damage was found as early as two weeks from starting treatment and all eight within 16 weeks. It was not possible to reach a conclusion on the mutagenicity of phenylbutazone due to the lack of adequate *in vivo* studies.
18. The carcinogenic potential of phenylbutazone was evaluated by the International Agency for Research on Cancer in 1987. The data available at that time was limited and considered insufficient to reach a conclusion. Since that time three studies have become available, including two GLP studies in rats and mice conducted for the US National Toxicology Program.

19. Inbred DONRYU rats were fed diet containing 0, 1250 or 2500 mg/kg feed phenylbutazone for 2 years. In females dose-dependent positive trends were seen for leukaemia, hepatic neoplasms and adrenal pheochromocytomas. Tumour-promoting effects of phenylbutazone were also investigated in rats. After initiation with *N*-ethyl-*N*-nitrosourea (ENU) or *N*-propyl-*N*-nitrosourea (PNU), animals received either basal diet or diet containing 2500 mg/kg feed phenylbutazone for 2 years. Rats exposed to *N*-ethyl-*N*-nitrosourea or *N*-propyl-*N*-nitrosourea showed increased incidences of ovarian neoplasms, leukaemia, gliomas, intestinal neoplasms and cortical neoplasms, incidences of these tumours were not increased by phenylbutazone exposure. However a slight promoting effect was noted for renal and thyroid tumours.
20. B6C3F₁ mice were given daily oral doses of phenylbutazone (0, 150, 300 mg/kg bw), 5 days/week for 103 weeks. No treatment-related clinical signs were reported and survival was unaffected. Mean bodyweights were slightly decreased in the high dose. Hepatocellular adenomas or hepatocellular adenomas and carcinomas combined showed significant positive trends in males and were significantly greater at the high dose, hepatocellular neoplasms were not increased in females. Non-neoplastic hepatocellular lesions also showed significant (and in some cases, dose-related) increases. A significant decrease in lymphomas was seen in high dose male mice compared to controls. No NOEL for neoplastic or non-neoplastic effects can be derived from this study.
21. F344/N rats were given daily oral doses of phenylbutazone (0, 50, 100 mg/kg bw), 5 days/week for 103 weeks. Survival of male low dose animals was statistically significantly lower than controls, survival of high dose females was slightly decreased. Bodyweights of the high dose were lower than controls. No adverse clinical effects were reported. Incidence and severity of nephropathy and renal lesions were significantly greater in both dose groups and both sexes than controls. Small numbers of renal tubular cell adenomas and carcinomas were seen in male rats and tubular cell adenomas and rare transitional cell carcinomas of the pelvic epithelium were seen in females. Adrenal gland medullary hyperplasia was significantly increased in the high dose females. Lesions of the forestomach were significantly increased in the treated males and high dose females. An increased incidence of histiocytic infiltration of the lung was seen in the high dose females and a statistically decreased incidence of mammary fibroadenomas was seen in the high dose females with a significant negative trend. No NOEL for neoplastic or non-neoplastic effects can be derived from this study.
22. These studies indicate that phenylbutazone is probably carcinogenic to male mice and female rats. There is some limited evidence that phenylbutazone can also act as a tumour promotor.
23. No experimental studies have been performed in animals to investigate potential immunotoxic effects of phenylbutazone. The data on adverse effects in humans indicates (idiosyncratic) reactions to the compound that have occasionally been fatal. As no mechanism has been established for the blood dyscrasias in humans and neutropenia was seen in some animal species, further investigation of the immunotoxic capability of phenylbutazone would be desirable.

Adverse reactions are commonly reported with clinical use of phenylbutazone in humans. Between 10-45% of patients report some type of side-effect, which cover a broad spectrum of conditions. The most serious adverse effects from phenylbutazone treatment in humans are agranulocytosis and aplastic anaemia. Leucopenia, pancytopenia, haemolytic anaemia and thrombocytopenia may also occur. Mortality from fatal bone marrow depression due to phenylbutazone and oxyphenbutazone is estimated at 2.2 and 3.8 per 100000, respectively, compared to a figure of around 2.5-5 per 100000 for chloramphenicol. In the UK phenylbutazone is currently only indicated for hospital use for cases of ankylosing spondylitis when other therapy has failed.

24. Inhibition of DNA synthesis in cultured human lymphocytes *in vitro* by short term exposure to therapeutic concentrations of phenylbutazone are reported. Inhibition occurred even at the lowest dose tested (32 μM). In the presence of serum, phenylbutazone only caused appreciable inhibition in one case (dose not specified). Inhibition of DNA synthesis in cultured human bone marrow cells aspirated from lung cancer patients has been investigated. In bone marrow cells from two patients (both male), phenylbutazone caused a dose-dependent decrease in uptake of $^3\text{H-TdR}$ that was negated by serum, in the third (female), a much greater inhibition occurred and there was still inhibition in the presence of serum at levels of phenylbutazone (30-600 μM) well within the therapeutic range. Significant but slight decreases in cell viability were seen with increasing doses but at only 6% in the highest doses were not considered to be responsible for the effect on DNA synthesis. It was also noted that inhibition was brought about by only one hour's exposure and the magnitude of inhibition in the absence of serum is comparable to that of cytotoxic drugs used in treatment of leukaemias, e.g. cytosine arabinoside and hydroxyurea. *In vitro* studies to investigate effects of phenylbutazone on growth of bone marrow cells have been performed using haematologically normal human granulocyte and monocyte cultures. Dose-dependent inhibition of colony formation was reported. Phenylbutazone had an ED_{50} around 180 μM . *In vivo*, peak plasma concentrations of phenylbutazone during treatment are in the region of 200-500 μM . Lymphocytes from fresh peripheral blood of nine healthy subjects were transformed to proliferating blastic forms and incubated in medium containing phenylbutazone at concentrations from 30-500 μM with $^3\text{H-TdR}$ for one hour, both with and without the presence of 20% serum. Phenylbutazone caused inhibition of DNA synthesis as measured by $^3\text{H-TdR}$ incorporation. Cell counts and trypan blue exclusion tests indicate these effects were not caused by lethal toxic effects. The inhibitory action of phenylbutazone, on *in vitro* growth of human granulocyte and monocyte colonies has also been investigated. Phenylbutazone was directly toxic to progenitor cells at concentrations around 250 μM . An ED_{50} value (concentration producing 50% inhibition of colony formation) was obtained of about 180 μM .
25. It is therefore concluded that a mechanism for phenylbutazone-induced myelotoxicity in humans has not yet been demonstrated. The clinical profile of phenylbutazone-induced dyscrasias is complicated. The *in vitro* investigations using haemopoietic progenitor cells indicate dose-dependent effects on DNA synthesis and chromosomal damage. However, it is difficult to relate the findings of these studies to each other, or to the events in phenylbutazone treated patients. Phenylbutazone-induced aplastic anaemia may show an insidious development after prolonged treatment, it may occur months after treatment has ceased, or occasionally develop acutely often on rechallenge. Agranulocytosis on the other hand appears more closely related to drug exposure and in many cases occurs within days of starting treatment. No animal model exists for these myelotoxic effects.
26. No data are provided on either the effects of phenylbutazone on human gut flora or micro-organisms used in food processing. Such data are not considered necessary for this class of substance.
27. Oxyphenbutazone and γ -hydroxyphenylbutazone are the principal metabolites and both are reported to exhibit anti-inflammatory activity. The available data on oxyphenbutazone was limited to reports of pharmacological studies, acute toxicity data and effects on the haemopoietic system. It is unclear whether phenylbutazone or oxyphenbutazone is the more potent substance in terms of pharmacological effects and toxicity to laboratory animals. However the data on myelotoxic effects suggest that oxyphenbutazone is more toxic than the parent compound.

28. It was concluded that no ADI could be established for the following reasons:
- The most critical known toxic effects of phenylbutazone are blood dyscrasias in humans. It is not possible to determine a NOEL for this effect. Phenylbutazone has been used for a range of indications in the past and blood dyscrasias have been reported over the range of therapeutic doses. The myelotoxic effects of phenylbutazone and its metabolites are analogous with the effects of chloramphenicol and cytotoxic drugs;
 - Phenylbutazone is nephrotoxic in the rat and hepatotoxic in the mouse. There is evidence of carcinogenicity in one or both sexes in both species. NOELs or minimum effect levels in these studies cannot be derived from these studies;
 - No adequate data on reproductive toxicity are available;
 - *In vitro* studies have shown evidence of mutagenic potential and examination of lymphocytes from human patients shows significant increases in chromosomal damage. No adequate studies to investigate the mutagenic activity of phenylbutazone *in vivo* have been conducted.
29. No radiometric studies were presented in the target species, hence no justification for the choice of a marker residue was given and no information concerning the ratio of marker to total residues for any of the target species was provided.
30. In pigs, intravenous doses of 20 and 40 mg phenylbutazone/kg bw gave phenylbutazone V_d values of 0.14 and 0.22 l/kg respectively. In the same plasma samples the V_d values of the metabolite oxyphenbutazone were 0.19 and 0.41 l/kg respectively. Elimination data were also presented for commercial phenylbutazone preparations in pigs (intramuscular 7 mg/kg bw; $t_{1/2}$ = 12.5 hours). The metabolites in 24 hour urine samples from pigs dosed intramuscularly could be ranked on the basis of concentration as follows: oxyphenbutazone greater than γ -hydroxy-phenylbutazone greater than $p\gamma$ -dihydroxyphenylbutazone and greater than phenylbutazone.
31. When ponies were dosed orally or intravenously with 4.4 mg phenylbutazone/kg bw (commercial formulation) the changes in plasma phenylbutazone concentrations with time fitted a two compartment open model. The plasma concentrations of phenylbutazone following oral administration indicated a mean bioavailability of 68% based on the AUC_{0-128h} . Elimination data were also presented for commercial phenylbutazone preparations in horses (intravenous 6 mg/kg bw; $t_{1/2}$ = 6.2 hours).
32. In cattle treated with 7.8 mg phenylbutazone/kg bw (commercial formulation containing ramifenazone), the highest tissue concentrations 8 hours after dosing were detected in plasma, edible tissues contained relatively lower residue concentrations (1.4 mg/kg in liver and 1.3 mg/kg in kidney).
33. In healthy and leukaemia virus infected cattle the elimination half-lives ($t_{1/2}$) following intravenous and oral administration of 2×5 mg phenylbutazone/kg bw were 31.4 to 78.2 hours (healthy to infected) and 36.8 to 78.2 hours (healthy to infected) respectively.
34. In cattle dosed twice with 5 mg/kg bw with a 3 week interval between doses, the variation in plasma concentrations with time fitted a three compartment open model and indicated a mean bioavailability of 68%. In cattle treated with 7.8 mg phenylbutazone/kg bw (commercial formulation containing ramifenazone), the area under the curve was 5221 mg.h/l. Plasma protein binding of phenylbutazone was found to be greater than 90% in all species tested.

35. The majority of the 45 pharmacokinetic studies presented by the applicants were extracted from the published literature. None of the studies presented were compliant with the requirements of GLP. The pharmacokinetic studies were performed using commercial preparations of phenylbutazone containing other pharmacologically active compounds (ramifenazone and/or dexamethasone), or used isopropylantipyrine (a compound closely related to phenylbutazone making critical comparison of the data impossible. When the target species were dosed with phenylbutazone the major metabolites in plasma and urine samples were phenylbutazone and oxyphenbutazone (by HPLC or GLC).
36. When pigs were intramuscularly dosed 21 mg phenylbutazone/kg bw with a commercial phenylbutazone preparation (containing ramifenazone and dexamethasone), all tissues at 6, 8 and 10 day timepoints contained residue concentrations of phenylbutazone, oxyphenbutazone and ramifenazone below 500 µg/kg (the analytical limit of detection). Other possible metabolites of phenylbutazone were not assayed for and residue concentrations in injection site tissues were not measured. The study did not comply with the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community in terms of the number of animals per time point.
37. In a pony 6 hours after oral dosing with 4.4 mg phenylbutazone/kg bw phenylbutazone and oxyphenbutazone concentrations were 700 µg/kg and 200 µg/kg respectively in liver, 700 µg/kg and 1100 µg/kg respectively in kidney and 100 µg/kg and 100 µg/kg respectively in muscle. Phenylbutazone and oxyphenbutazone residue concentrations in a pony killed 24 hours after dosing were 200 µg/kg and less than 100 µg/kg respectively in liver, 400 µg/kg and 400 µg/kg respectively in kidney and less than 100 µg/kg and less than 100 µg/kg respectively in muscle. Plasma residue concentrations over the same time period fell from 3.3 µg/ml and 1.1 µg/ml respectively to 0.600 µg/ml and 0.2 µg/ml respectively. No results for residue concentrations in the fat tissues of ponies after dosing with phenylbutazone were presented. Higher residues were found in a study in horses with one animal slaughtered per time point; however the dosage regime for this study was not given.
38. When cows were intravenously injected with 7.5 mg phenylbutazone/kg bw, phenylbutazone concentrations in biopsy samples of liver and muscle were highest (8400 µg/kg and 5600 µg/kg respectively) 7 hours after dosing (second timepoint). These concentrations depleted to below the limit of detection (less than 50 µg/kg actual value not given) by 392 hours (ca. 17 days) after dosing. No kidney or fat samples were analysed. In another study using intravenous injection of 7.8 mg/kg bw phenylbutazone (+ 14.4 mg/kg bw metamizole) and 2 animals per time point residues of phenylbutazone in liver and kidney were 1420 and 1380 µg/kg, 192 hours after dosing and less than 500 and 800 µg/kg 240 hours after dosing. Residues in fat samples were below 500 µg/kg (the limit of detection). None of the cattle studied complied with the requirements of Volume VI in terms of numbers of animals per time point.
39. In 6 lactating cows 1 hour after intravenous dosing 7.8 mg phenylbutazone/kg bw mean residue concentrations of phenylbutazone and oxyphenbutazone in milk were 5 µg/kg and 64 µg/kg respectively. These concentrations had depleted to below the limit of detection (5 µg/kg) in all samples by 24 hours for phenylbutazone and 48 hours for oxyphenbutazone.
40. None of the residue depletion studies presented meet the requirements of Volume VI. It was not possible to assess the suitability of phenylbutazone or phenylbutazone+oxyphenbutazone as the marker residue in food producing species due to the lack of data. The ratio of these marker residues to total tissue residue was not known for any of the target species. Additionally, metabolism data in rats and humans suggest that phenylbutazone residues represent only a very minor fraction (less than 5%) of urinary residues and that metabolites such as p-γ-dihydroxyphenylbutazone or γ-hydroxyphenylbutazone could be better choices for a marker residue. Furthermore, there was evidence of rapid phenylbutazone degradation in muscle tissue homogenates.

41. A number of analytical methods were provided for the determination of residues of phenylbutazone or phenylbutazone+oxyphenylbutazone as the marker residue in sample matrices from food producing species. Most methods were based on HPLC. None of the methods was validated in accordance with the requirements of Volume VI.

Conclusions

Having considered that no information was provided in answer to the list of questions regarding:

- the pharmacological and toxicological NOELs,
- the submission of adequate reproduction toxicity and teratogenicity studies,
- the submission of adequate mutagenicity studies,
- the mechanism of the carcinogenic potential of phenylbutazone observed in laboratory animals,
- the aetiology for the blood dyscrasias observed in humans arising from clinical use of phenylbutazone and a threshold level for these effects,
- radiometric studies demonstrating residue depletion and the ratio of marker residue to total residue,
- the choice of the marker residue,
- a routine analytical method for monitoring purposes,

and that no ADI could be established and therefore no MRLs could be elaborated,

the Committee for Veterinary Medicinal Products concluded that a recommendation for the inclusion of phenylbutazone into any of the annexes of Council Regulation (EEC) No 2377/90 cannot be made.

B. Exposure assessment

B1 *Phenylbutazone in horse samples*

- ✓ Data from National Residue Control Plans

The EU Member States (MS) routinely monitor residues of veterinary medicinal product in live animals and animal products under the provision of the Council Directive 96/23/EC⁴. In this framework, most of the MS ((22/27) are looking for phenylbutazone in horse samples as part of their National Residue Control Plans (NRCP); the few countries that are not monitoring phenylbutazone producing less than 3 % of the total EU horse production.

Results from the NRCP of 19 Member States were collated in the framework of this scientific statement, representing a total of 2 386 samples taken from years 2005 to 2013 (Table B1). The samples were mostly taken at the slaughterhouse. Different matrices were tested: kidney and liver (1,171 samples), muscle (672 samples), serum and plasma (475 samples), other (milk, urine: 10 samples). Overall, 37 samples (1.6 %) were found positive for phenylbutazone. Kidney was the matrix with both the highest detection rate (2.9 % corresponding to 33 out of 1 160 kidney samples) and highest levels measured: from traces up to 1 900 µg/kg, with a median at 4.0 µg/kg. Two samples of plasma and serum (0.4 %) were found positive for phenylbutazone with levels at 3 and 6.5 µg/kg. Only one sample of muscle out of 672 (0.1 %) was found positive, with phenylbutazone at 19.2 µg/kg.

A limitation of this dataset is represented by the high inconsistency present both in the analysed matrices and the sampling strategies. In particular it is noted that the presence of phenylbutazone is monitored in different matrices including kidney, liver, serum, plasma and muscle. Regarding the sampling strategy, a substantial variability of the sample proportion was observed among different Member States with in some cases an extremely low number of samples. Finally, some differences of around a factor 10 were observed in the limits of quantification or CC_{alpha} reported for the different matrices.

In addition to these data, it has to be pointed out that in the framework of their NRCP, some countries have also found phenylbutazone in beef samples. The EFSA reports on the results from the monitoring of veterinary medicinal product residues and other substances in live animals and animal products mention that 17 beef samples were found positive to phenylbutazone during the 2009 – 2011 period (EFSA, 2011a, 2012, 2013).

- ✓ Information from the Rapid Alert System for Food and Feed (RASFF)

The presence of phenylbutazone has also been notified to the Rapid Alert System for Food and Feed (RASFF) in horse products imported into the European Union (EU). In 2012, phenylbutazone was found by the Canadian authorities at 10 µg/kg in kidney and 1.3 µg/kg in muscle tissue of a horse, for which some derived products have been imported into the EU (RASFF notification n°2012.1078).

The presence of phenylbutazone has been reported in frozen beef burgers and meatballs taken from the market in Portugal at levels of 2 and 11 µg/kg. These samples were also found positive for horse DNA but quantification gave less than 1 % of horse DNA (RASFF notification n°2013.0445).

⁴ Council Directive 96/23/EC of 29 April 1996 on measures to mo

Table B1: Results from the monitoring of phenylbutazone in horse samples throughout Europe.

Member State	Average annual production ^(a)	Monitoring period	Total number of samples tested	Total number of positive samples (%)	Matrices analysed	Analytical technique	LOQ or CCalpha (µg/kg)	Quantified values (µg/kg)
Austria	943	na	Na	na	Muscle	na	na	
Belgium	12,000	2009-2012	132	0	Muscle	LC-MS/MS	2 - 10	
Bulgaria	2,667	2011	8	0	Muscle	na	11.3	
Cyprus	6,800	-*	-*	-*	-*	-*	-*	
Czech Republic	305	2010-2012	24	1 (4.2)	Muscle	HPLC-MS	2.5	19.2
Denmark	2,454	2008-2012	33	1 (3.0)	Plasma	LC-MS/MS, HPLC-DAD	2 - 13.6	3
Estonia	0	-*	-*	-*	-*	-*	-*	
Finland	1,102	2008-2012	30	0	Muscle	LC-MS/MS	3.2	
France	16,446	2008-2012	329	0	Muscle	LC-MS/MS	0.92	
Germany	9,109	2005-2012	188	1 (0.5)	Liver, plasma, serum, muscle, kidney, milk, urine	na	na	8.2 (liver)
Greece	0	-*	-*	-*	-*	-*	-*	
Hungary	173	2010-2012	3	0	Liver, muscle, plasma	na	na	
Ireland	4,291	2005-2012	361	1 (0.3)	Kidney	na	5	10
Italy	94,375	2009-2011	47	0	Plasma	na	na	
Latvia	418	2010-2012	7	0	Muscle	na	na	
Lithuania	2,205	na	Na	na	Muscle	na	na	
Luxembourg	0	-*	-*	-*	-*	-*	-*	
Malta	131	na	Na	na	Kidney	na	na	
The Netherlands	1,992	2005-2012	36	0	Muscle	na	na	
Poland	43,651	2009-2011	92	0	Muscle	na	3.8	
Portugal	1,044	2010-2012	9	0	Muscle	na	na	
Romania	20,651	2011	2	0	Muscle	na	1.66	
Slovakia	11	-*	-*	-*	-*	-*	-*	
Slovenia	1,494	2010-2012	6	0	Plasma	na	26	
Spain	28,562	2008-2012	58	0	Muscle	na	na	
Sweden	3,724	2010-2012	225	1 (0.4)	Serum	LC-MS/MS	2.5	6.5
United Kingdom	4,175	2009-2013	796	32 (4.0)	Kidney	na	na	Traces x5, 0.84, 1.0x2, 1.1x2, 1.4, 1.6, 2.0, 2.3, 2.5, 2.8, 3.8, 4.1, 4.9, 5, 5.3, 6.4, 6.5, 8, 8.2, 10, 12,

Member State	Average annual production ^(a)	Monitoring period	Total number of samples tested	Total number of positive samples (%)	Matrices analysed	Analytical technique	LOQ or CCalpha (µg/kg)	Quantified values (µg/kg)
								49, 110, 1200x2, 1900
European Union	303,067		2,386	37 (1.6)				

na: data not available; LC-MS/MS: liquid chromatography – tandem mass spectrometry; HPLC: high performance liquid chromatography; MS: mass spectrometry; DAD: diode array detection; LOQ: limit of quantification.

*(a) : annual production: average number of animals produced during the period 2008-2010 (EFSA, 2011a, 2012, 2013). *: Phenylbutazone not tested in horse meat. na: data not available.*

B2 Oxyphenbutazone in horse samples

This metabolite of phenylbutazone is monitored by some MS in the framework of their NRCP. The EFSA reports on the results from the monitoring of veterinary medicinal product residues and other substances in live animals and animal products mention eight positive samples found during the 2009 – 2011 period (EFSA, 2011a, 2012, 2013). Seven of them were corresponding to horse blood taken in live animals during a local competition in Belgium in 2010 and were not considered as relevant for the analysis. The last sample corresponded to horse muscle taken in Czech Republic in 2011. Oxyphenbutazone and phenylbutazone were respectively found at 57.1 and 19.3 µg/kg in that sample.

B3 Data on the presence of horse meat in beef products

Two analytical techniques are currently available to identify and quantify meat species: the polymerase chain reaction (PCR), which is based on the detection and quantification of horse DNA, and the Enzyme-Linked Immunosorbent Assay immunological (ELISA), which is based on the detection and quantification of horse proteins. In the further paragraphs, the term ‘horse meat’ used without further details refers either to horse DNA or horse proteins. Moreover, the percentage of equine DNA relative to bovine DNA is considered equivalent to the percentage of horse meat related to the total meat content. According to the European Commission Recommendation (2013/99/EU)⁵, a working limit of 1 % undeclared ‘horse meat’ in a food containing beef triggers further investigation of possible adulteration. Indeed, levels below this working limit might result from cross-contamination. Some results were reported according to such working limit whereas some other were reported in regards to the limit of detection/quantification.

B3.1. Results from industry tests

According to the third summary report on results of industry testing of meat products published by the UK FSA on the 1st March 2013, around 1 % of the beef-based products tested (44/4,196 samples) contained more than 1 % horse meat (UK-FSA, 2013a). Horse meat was not found in other (non-beef) meat products (223 samples tested). The beef based products found positive for horse meat corresponded to: pasta with meat (lasagne, tortellini, etc...), meat sauce (sauce Bolognese), meat burgers, minced/ground beef meat.

According to the reports of industry tests for horse meat published by the Food Safety Authority of Ireland on the 1st and 25th March 2013, horse meat was found above 1 % in 31 out of 1,856 (1.7 %) beef-based products tested (FSAI, 2013a,b). The beef-based products found positive corresponded to pasta with meat (lasagne, spaghetti Bolognese), beef burgers and meatballs, meatloaf and cottage pie. Horse meat was found to represent up to 30 % of the meat content in burgers, up to 100 % of the meat content in pasta with meat (FSAI, 2013c,d) and up to 5.2 % of the meat content in the other products. Horse meat was not found in beef meat ingredients (429 samples tested).

B3.2. Results from Member States surveys

The preliminary results of the UK FSA’s UK wide survey of beef products, as published by the UK FSA on the 26th of March 2013 (UK-FSA, 2013b), aimed to be representative of the products available across UK, showed that 2 samples out of 362 were found to contain horse DNA above 1 %. At the time of the publication, three samples were

⁵ Commission Recommendation of 19 February 2013 on a coordinated control plan with a view to establish the prevalence of fraudulent practices in the marketing of certain foods. OJ L 48, 21.2.2013, p. 28-32.

still undergoing further investigations, as their results were disputed by the food business or manufacturer. The two positive samples corresponded to meat burger and meat balls.

B3.3 Information from the Rapid Alert System for Food and Feed

On the 15th March 2013, 37 notifications of adulteration of beef based products with horse meat were received by the RASFF originating from 13 MS. Around one quarter of the notifications corresponded to pasta with meat. The other beef based products found positive more than once were meat burgers, meat balls, beef goulash, beef stewed and frozen pieces of beef. The presence of horse meat was also notified in sauce Bolognese, chilli con carne, corned beef, canned beef, cottage pie, beef wraps, beef kebab, minced beef and frozen sausage. The percentage of horse meat measured represented up to 90 % of the meat content in burgers and meatballs, 80 % of the meat stewed, 100 % of the meat content in pasta with meat and 70 % of the meat content in the other beef based products.

B4 Consumption data of horse and beef based products

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database) is a source of information on food consumption across the European Union (EFSA, 2011b). It gathers detailed data originated from 32 national food consumption surveys representing 66,492 individuals of 22 EU Member States covering all age groups (infant, toddlers, other children, adolescents, adults, elderly, very elderly).

B4.1 Consumption habits of horse products

The consumption levels available for horse meat are detailed for the different countries and age groups in the Table B2.

Consumption of horse meat and/or horse meat products (sausages, preserved meat) was reported in 9 out of 22 countries: France, Italy, Belgium, Sweden, Spain, the Netherlands, Germany, Slovenia and Slovakia. Whatever the country and the age class considered, the number of consumers of horse meat was very low: less than 10 individuals in 20 population groups, between 11 and 53 individuals in 8 population groups. The maximum percentage of horse meat consumers estimated across the population groups was estimated to be approximately 3 %.

From the surveys with at least 7 reporting days, the frequency of horse meat consumption among the consumers only was estimated to be on average approximately 1.5 times per week, with a the 95th percentile of two times per week.

From the surveys with at least 5 horse meat consumption events (i.e. days of horse meat consumption), the average daily portion size ranged across the population groups between 16.9 and 85.5 g for children up to 10 years old and between 30.9 to 152.4 g for adults. Due to the very low number of consumption events, the high daily portion size consumed – i.e. 95th percentile – could be characterised only for French and Italian adults at 200.0 g (N = 61 consuming days) and 252.0 g (N = 60 consuming days) respectively.

No consumption event of horse offal was reported in the Comprehensive Database. It is assumed that horse offal would not be consumed as such in Europe or at very infrequent occasions.

Table B2: Horse meat consumption data from the Comprehensive Database.

Dietary survey	Country	Number of individuals	Number of horse meat consumers (%)	Number of reporting days	Average frequency of horse meat consumption per week (P95)	Number of consuming days	Daily portion size			
							Average		P95	
							g	g/kg b.w.	g	g/kg b.w.
Toddlers (≥ 1 year to < 3 years old)										
Regional_Flanders	Belgium	36	1 (2.8)	3	-	1	-	-	-	-
NUTRICHILD	Bulgaria	428	0 (0)	-	-	-	-	-	-	-
DIPP	Finland	500	0 (0)	-	-	-	-	-	-	-
DONALD 2006, 2007, 2008	Germany	261	0 (0)	-	-	-	-	-	-	-
INRAN_SCAI_2005_06	Italy	36	0 (0)	-	-	-	-	-	-	-
VCP_kids	The Netherlands	322	0 (0)	-	-	-	-	-	-	-
IZZ_FAO_2000	Poland	79	0 (0)	-	-	-	-	-	-	-
enKid	Spain	17	0 (0)	-	-	-	-	-	-	-
Other children (≥ 3 years to < 10 years old)										
Regional_Flanders	Belgium	625	8 (1.3)	3	-	8	67.3	3.9	-	-
NUTRICHILD	Bulgaria	434	0 (0)	-	-	-	-	-	-	-
SISP04	Czech Republic	389	0 (0)	-	-	-	-	-	-	-
Danish_Dietary_Survey	Denmark	490	0 (0)	-	-	-	-	-	-	-
DIPP, STRIP	Finland	1,183	0 (0)	-	-	-	-	-	-	-
INCA2	France	482	9 (1.9)	7	1.2 (-)	11	85.5	3.8	-	-
DONALD 2006, 2007, 2008	Germany	660	0 (0)	-	-	-	-	-	-	-
Regional_Crete	Greece	847	0 (0)	-	-	-	-	-	-	-
INRAN_SCAI_2005_06	Italy	193	1 (0.5)	3	-	1	-	-	-	-
EFSA_TEST	Latvia	190	0 (0)	-	-	-	-	-	-	-
VCP_kids	The Netherlands	957	2 (0.2)	2	-	2	-	-	-	-
IZZ_FAO_2000	Poland	409	0 (0)	-	-	-	-	-	-	-
NUT_INK05	Spain	399	2 (0.5)	2	-	3	-	-	-	-
enKid	Spain	156	1 (0.6)	2	-	1	-	-	-	-
NFA	Sweden	1,473	15 (1.0)	4	-	18	16.9	0.8	-	-
Adolescents (≥ 10 years to < 18 years old)										
Diet_National_2004	Belgium	611	6 (1.0)	2	-	6	144.8	2.6	-	-

Dietary survey	Country	Number of individuals	Number of horse meat consumers (%)	Number of reporting days	Average frequency of horse meat consumption per week (P95)	Number of consuming days	Daily portion size			
							Average		P95	
							g	g/kg b.w.	g	g/kg b.w.
NSFIN	Bulgaria	162	0 (0)	-	-	-	-	-	-	-
Childhealth	Cyprus	303	0 (0)	-	-	-	-	-	-	-
SISPO4	Czech Republic	298	0 (0)	-	-	-	-	-	-	-
Danish_Dietary_Survey	Denmark	479	0 (0)	-	-	-	-	-	-	-
INCA2	France	973	21 (2.2)	7	1.2 (-)	26	130.8	2.6	-	-
National_Nutrition_Survey_II	Germany	1,011	0 (0)	-	-	-	-	-	-	-
INRAN_SCAI_2005_06	Italy	247	7 (2.8)	3	-	7	118.1	2.4	-	-
EFSA_TEST	Latvia	496	0 (0)	-	-	-	-	-	-	-
IZZ_FAO_2000	Poland	666	0 (0)	-	-	-	-	-	-	-
NUT_INK05	Spain	651	1 (0.2)	2	-	1	-	-	-	-
enKid	Spain	209	2 (1.0)	2	-	2	-	-	-	-
AESAN_FIAB	Spain	86	0 (0)	-	-	-	-	-	-	-
NFA	Sweden	1,018	9 (0.9)	4	-	13	30.9	0.9	-	-
Adults (≥ 18 years to < 65 years old)										
ASNS	Austria	2,123	0 (0)	-	-	-	-	-	-	-
Diet_National_2004	Belgium	1,356	16 (1.2)	2	-	18	152.4	1.9	-	-
NSFIN	Bulgaria	691	0 (0)	-	-	-	-	-	-	-
SISPO4	Czech Republic	1,666	0 (0)	-	-	-	-	-	-	-
Danish_Dietary_Survey	Denmark	2,822	0 (0)	-	-	-	-	-	-	-
NDS_1997	Estonia	1,866	0 (0)	-	-	-	-	-	-	-
FINDIET_2007	Finland	1,575	0 (0)	-	-	-	-	-	-	-
INCA2	France	2,276	52 (2.3)	7	1.2 (2.2)	61	139.3	2.0	200.0	3.5
National_Nutrition_Survey_II	Germany	10,419	12 (0.1)	2	-	12	118.5	1.3	-	-
National_Repr_Surv	Hungary	1,074	0 (0)	-	-	-	-	-	-	-
NSIFCS	Ireland	958	0 (0)	-	-	-	-	-	-	-
INRAN_SCAI_2005_06	Italy	2,313	57 (2.5)	3	-	60	122.6	1.7	252.0	3.7
EFSA_TEST	Latvia	1,384	0 (0)	-	-	-	-	-	-	-
DNFCS_2003	The Netherlands	750	2 (0.3)	2	-	2	-	-	-	-

Dietary survey	Country	Number of individuals	Number of horse meat consumers (%)	Number of reporting days	Average frequency of horse meat consumption per week (P95)	Number of consuming days	Daily portion size			
							Average		P95	
							g	g/kg b.w.	g	g/kg b.w.
IZZ_FAO_2000	Poland	2,527	0 (0)	-	-	-	-	-	-	
SK_MON_2008	Slovakia	2,761	1 (0.0)	1	-	1	-	-	-	
CRP_2008	Slovenia	407	1 (0.2)	1	-	1	-	-	-	
AESAN, AESAN-FIAB	Spain	1,391	0 (0)	-	-	-	-	-	-	
Riksmaten_1997_98	Sweden	1,210	8 (0.7)	7	1.6	13	40.0	0.6	-	-
NDNS	United Kingdom	1,724	0 (0)	-	-	-	-	-	-	-
Elderly (≥ 65 years to < 75 years old)										
Diet_National_2004	Belgium	534	11 (2.1)	2	-	12	86.6	1.2	-	-
NSFIN	Bulgaria	151	0 (0)	-	-	-	-	-	-	-
Danish_Dietary_Survey	Denmark	309	0 (0)	-	-	-	-	-	-	-
FINDIET_2007	Finland	463	0 (0)	-	-	-	-	-	-	-
INCA2	France	264	8 (3.0)	7	1.0	8	134.4	1.9	-	-
National_Nutrition_Survey_II	Germany	2,006	2 (0.1)	2	-	-	-	-	-	-
National_Repr_Surv	Hungary	206	0 (0)	-	-	-	-	-	-	-
INRAN_SCAI_2005_06	Italy	290	11 (3.8)	3	-	15	73.8	1.0	-	-
IZZ_FAO_2000	Poland	329	0 (0)	-	-	-	-	-	-	-
Very elderly (≥ 75 years old)										
Diet_National_2004	Belgium	744	8 (1.1)	2	-	9	126.6	1.9	-	-
NSFIN	Bulgaria	200	0 (0)	-	-	-	-	-	-	-
Danish_Dietary_Survey	Denmark	20	0 (0)	-	-	-	-	-	-	-
INCA2	France	84	0 (0)	-	-	-	-	-	-	-
National_Nutrition_Survey_II	Germany	490	0 (0)	-	-	-	-	-	-	-
National_Repr_Surv	Hungary	80	0 (0)	-	-	-	-	-	-	-
INRAN_SCAI_2005_06	Italy	228	4 (1.8)	3	-	4	94.3	1.7	-	-
IZZ_FAO_2000	Poland	124	0 (0)	-	-	-	-	-	-	-

B4.2 Consumption habits of beef-based products

Consumption of beef-based products (pasta filled with meat, meat burgers and balls, minced beef meat, beef canned, beef corned, beef goulash, beef kebab, beef loaf, beef stewed, beef sausage, meat pie, sauce Bolognese and chilli con carne) was reported in almost all countries (21/22 countries) (Table B3). The percentage of consumers of beef-based products was higher in the groups of children and adolescents (up to 92 % consumers according to the survey) than among the groups of adults and elderly (up to 65 % consumers according to the survey).

From the surveys with at least 7 reporting days, the frequency of beef-based products consumption among consumers only was estimated on average around 2 times per week, and at the 95th percentile around 4 times per week.

Table B4 summarizes the daily portion sizes estimated for the meat content of different beef-based products. In the children population groups, the highest portion sizes corresponded to meat burgers and meat balls (80 % meat), which ranged across the population group on average between 33.2 and 142.4 g and between 84.0 and 224.0 g for the 95th percentile. In the adult population groups, the highest average portion sizes corresponded to beef stewed (80 % meat), which ranged across the population groups between 43.1 and 203.8 g. The highest high (95th percentile) portion sizes corresponded to minced/ground beef meat (100 % meat), which ranged between 129.6 and 300.0 g.

Table B3: Number of consumers and frequency of consumption of beef-based products in the Comprehensive database.

Dietary survey	Country	Number of individuals	Number of beef products ^(a) consumers (%)	Number of reporting days	Average frequency of beef products consumption per week (P95)
Toddlers ((≥ 1 year to < 3 years old))					
Regional_Flanders	Belgium	36	27 (75.0)	3	-
NUTRICHILD	Bulgaria	428	125 (29.2)	-	-
DIPP	Finland	500	242 (48.4)	3	-
DONALD 2006, 2007, 2008	Germany	261	73 (28.0)	3	-
INRAN_SCAI_2005_06	Italy	36	1 (2.8)	3	-
VCP_kids	The Netherlands	322	120 (37.3)	2	-
IZZ_FAO_2000	Poland	79	29 (36.7)	-	-
enKid	Spain	17	1 (5.9)	-	-
Other children (≥ 3 years to < 10 years old)					
Regional_Flanders	Belgium	625	454 (72.6.9)	3	-
NUTRICHILD	Bulgaria	434	133 (30.6)	-	-
SISP04	Czech Republic	389	56 (14.4)	2	-
Danish_Dietary_Survey	Denmark	490	0 (0)	-	-
DIPP	Finland	948	698 (73.6)	3	-
STRIP	Finland	250	230 (92.0)	4	-
INCA2	France	482	389 (80.7)	7	2.0 (4.0)
DONALD 2006, 2007, 2008	Germany	660	200 (30.3)	3	-
Regional_Crete	Greece	847	372 (43.9)	3	-
INRAN_SCAI_2005_06	Italy	193	6 (3.1)	3	-
EFSA_TEST	Latvia	190	58 (30.5)	2	-
VCP_kids	The Netherlands	957	309 (32.3)	2	-
IZZ_FAO_2000	Poland	409	120 (29.3)	-	-
NUT_INK05	Spain	399	5 (1.3)	2	-
enKid	Spain	156	34 (21.8)	2	-

Dietary survey	Country	Number of individuals	Number of beef products ^(a) consumers (%)	Number of reporting days	Average frequency of beef products consumption per week (P95)
NFA	Sweden	1,473	1,318 (89.5)	4	-
Adolescents (≥ 10 years to < 18 years old)					
Diet_National_2004	Belgium	611	270 (44.2)	2	-
NSFIN	Bulgaria	162	33 (20.4)	-	-
Childhealth	Cyprus	303	143 (47.2.0)	3	-
SISP04	Czech Republic	298	54 (18.1.7)	2	-
Danish_Dietary_Survey	Denmark	479	0 (0)	-	-
INCA2	France	973	773 (79.4)	7	2.0 (4.0)
National_Nutrition_Survey_II	Germany	1,011	334 (33.0)	2	-
INRAN_SCAI_2005_06	Italy	247	18 (7.3)	3	-
EFSA_TEST	Latvia	496	121 (24.4)	2	-
IZZ_FAO_2000	Poland	666	236 (35.4)	-	-
NUT_INK05	Spain	651	39 (18.7)	2	-
enKid	Spain	209	12 (5.7)	2	-
AESAN_FIAB	Spain	86	41 (47.7)	3	-
NFA	Sweden	1,018	874 (85.9)	4	-
Adults (≥ 18 years to < 65 years old)					
ASNS	Austria	2,123	308 (14.5)	1	-
Diet_National_2004	Belgium	1,356	497 (36.7)	2	-
NSFIN	Bulgaria	691	116 (16.8)	-	-
SISP04	Czech Republic	1,666	239 (14.3)	2	-
Danish_Dietary_Survey	Denmark	2,822	0 (0)	-	-
NDS_1997	Estonia	1,866	444 (23.8)	1	-
FINDIET_2007	Finland	1,575	615 (39.0)	2	-
INCA2	France	2,276	1,480 (65.0)	7	1.9 (4.0)
National_Nutrition_Survey_II	Germany	10,419	2,999 (28.8)	2	-
National_Repr_Surv	Hungary	1,074	37 (3.4)	3	-

Dietary survey	Country	Number of individuals	Number of beef products ^(a) consumers (%)	Number of reporting days	Average frequency of beef products consumption per week (P95)
NSIFCS	Ireland	958	528 (55.1)	7	1.8 (4.0)
INRAN_SCAI_2005_06	Italy	2,313	101 (4.4)	3	-
EFSA_TEST	Latvia	1,384	270 (19.5)	2	-
DNFCS_2003	The Netherlands	750	313 (41.7)	2	-
IZZ_FAO_2000	Poland	2,527	1044 (41.3)	-	-
SK_MON_2008	Slovakia	2,761	176 (6.4)	1	-
CRP_2008	Slovenia	407	3 (0.7)	1	-
AESAN	Spain	418	126 (30.1)	2	-
AESAN-FIAB	Spain	982	424 (43.2)	3	-
Riksmaten_1997_98	Sweden	1,210	651 (53.8)	7	1.6 (4.0)
NDNS	United Kingdom	1,724	989 (57.4)	7	1.9 (4.0)
Elderly (≥ 65 years to < 75 years old)					
Diet_National_2004	Belgium	534	137 (25.7)	2	-
NSFIN	Bulgaria	151	12 (7.9)	-	-
Danish_Dietary_Survey	Denmark	309	0 (0)	-	-
FINDIET_2007	Finland	463	122 (26.3)	2	-
INCA2	France	264	101 (38.3)	7	1.6 (3.0)
National_Nutrition_Survey_II	Germany	2,006	487 (24.3)	2	-
National_Repr_Surv	Hungary	206	1 (0.5)	3	-
INRAN_SCAI_2005_06	Italy	290	16 (5.5)	3	-
IZZ_FAO_2000	Poland	329	114 (34.7)	-	-
Very elderly (≥ 75 years old)					
Diet_National_2004	Belgium	712	172 (23.1)	2	-
NSFIN	Bulgaria	200	12 (6.0)	-	-
Danish_Dietary_Survey	Denmark	20	0 (0)	-	-
INCA2	France	84	37 (44.0)	7	1.4 (2.4)
National_Nutrition_Survey_II	Germany	490	108 (22.0)	2	-

Dietary survey	Country	Number of individuals	Number of beef products ^(a) consumers (%)	Number of reporting days	Average frequency of beef products consumption per week (P95)
National_Repr_Surv	Hungary	80	1 (1.3)	-	-
INRAN_SCAI_2005_06	Italy	228	13 (5.7)	3	-
IZZ_FAO_2000	Poland	124	32 (25.8)	-	-

P95: 95th percentile.

a: the beef-based products taken into account are those for which the presence of horse meat has been notified at least once at the European level: pasta filled with meat, meat burgers and balls, minced beef meat, beef canned, beef corned, beef goulash, beef kebab, beef loaf, beef stewed, beef sausage, meat pie, sauce Bolognese and chilli con carne.

Table B4: Range of daily portion sizes of beef meat contained in beef-based products estimated from the Comprehensive database.

Beef products	Children (up to 10 years old)						Adults (from 10 years old)					
	Average			95 th percentile			Average			P95		
	N	g	g/kg b.w.	N	g	g/kg b.w.	N	g	g/kg b.w.	N	g	g/kg b.w.
<i>Beef canned</i>	-	-	-	-	-	-	5	43.4 – 144.2	0.8 – 1.6	-	-	-
<i>Beef goulash</i>	1	42.6	1.8	-	-	-	7	46.7 – 109.7	0.8 – 1.5	2	147.4 – 202.9	2.1 – 2.6
<i>Beef kebab</i>	-	-	-	-	-	-	2	117.1 – 159.1	2.3	-	-	-
<i>Beef loaf</i>	2	53.9 – 58.4	1.8 – 2.2				2	52.4 – 57.8	0.7 – 0.8			
<i>Beef sausage</i>	3	37.7 – 43.9	1.6 –	2	100.0 –	5.6 – 6.5	21	24.8 –	0.3 –	8	91.0 –	1.2 –

			3.5		118.8			131.3	1.9		240.0	3.6
<i>Beef stewed</i>	3	48.6 – 95.6	2.0 – 3.9	1	120.0	4.8	12	43.1 – 203.8	0.6 – 2.8	3	138.0 – 190.0	2.2 – 3.5
<i>Chilli con carne</i>	-	-	-	-	-	-	1	103.4	1.5	-	-	-
<i>Corned beef</i>	-	-	-	-	-	-	5	18.8 – 77.6	0.3 – 1.0	3	91.0 – 172.0	1.1 – 2.1
<i>Meat burger</i>	1 1	41.4 – 142.4	2.4 – 5.7	3	90.4 – 168.0	5.0 – 6.2	24	45.0 – 154.7	0.9 – 2.6	9	95.2 – 288.0	1.7 – 5.3
<i>Meat pie</i>	1	64.5	2.4				3	61.7 – 98.2	0.8 – 2.7	-	-	-
<i>Meat balls</i>	1 3	33.2 – 98.3	1.7 – 4.5	4	84.0 – 224.0	3.9 – 9.1	13	51.7 – 132.7	0.8 – 2.4	4	120.0 – 200.0	1.8 – 4.2
<i>Minced/grounded beef</i>	1 8	17.2 – 87.7	1.5 – 3.9	1 0	79.0 – 165.0	4.1 – 7.9	31	57.8 – 155.1	0.7 – 2.4	22	129.6 – 300.0	1.7 – 4.8
<i>Pasta with meat</i>	5	19.8 – 43.6	0.7 – 2.0	3	60.0 – 90.0	2.3 – 3.9	14	17.8 – 46.5	0.3 – 0.9	4	59.3 – 75.0	1.0 – 1.6
<i>Sauce bolognese</i>	4	4.2 – 22.2	0.1 – 2.8	1	40.0	1.7	7	16.8 – 51.7	0.3 – 0.7	6	30.0 – 102.4	0.5 – 1.3

N = number of population groups; *b.w.*: body weight; *P95*: 95th percentile.

Note that the average daily portion size is derived from at least 5 consumption events in a survey, and the 95th percentile from at least 60 consumption events in a survey. The cooked beef meat was estimated to represent 15 % of pasta filled with meat (i.e. lasagne, tortellini), 20 % of meat sauce, 80 % of meat burgers, meat balls and meat loaf, 40 % of beef kebab, beef stewed, beef goulash, meat pie and chilli con carne, 100 % of minced/grounded meat, beef sausage, beef canned, beef corned.

B5 Likelihood of dietary exposure to phenylbutazone

The likelihood of dietary exposure to phenylbutazone was estimated at the consumer level by combining the frequency of consumption of horse meat or beef-based products potentially adulterated with horse meat with the prevalence of phenylbutazone in horse meat. An estimate of the number of individuals per 100 million potentially exposed each day to phenylbutazone was then derived at the population level by combining the likelihood of exposure at the individual level together with the estimated number of consumers of horse meat or beef meat products within the population. Calculations were done for each subgroup of population (country x age class combinations) with at least one consumer. Taking the uncertainty around the input parameters into account, different scenarios were considered (Table B5):

- Prevalence of phenylbutazone in horse meat: at 0.13% and 3.5 %. In absence of data related to the presence of phenylbutazone in horse meat as available on the EU market, the results from the NRCP on the presence of phenylbutazone in horse carcasses produced in Europe were used. Considering that in the framework of the global European market, a positive horse sample found at the production level in one country may have been associated with horse meat products found in the market of other countries, one single estimate was determined in order to represent the whole European market. 0.13 % corresponds to the average between the percentages of positive samples observed in the different countries weighted by the average annual production in the corresponding countries (Table B1). This scenario relies on the idea the results of the NRCP provide a true picture on the percentage of positive samples, even for the countries where a very low number of samples have been tested in regards to their production levels. A statistical analysis performed in order to assess the uncertainty around the prevalence of horse carcasses containing phenylbutazone in the EU showed that due to the low number of samples available in some countries, the prevalence at the EU level would be below 7.2 % with a 95 % confidence level (see Appendix D). The UK is among the Member States which reported the highest detection rates of phenylbutazone in horse samples in the last years, with a 3.5 % percentage of positive samples (17/480) since the beginning of 2013 in the framework of the UK-Food Standards Agency (FSA) '100% testing of horse carcasses'. This prevalence of 3.5 % was retained as a high estimate for the EU prevalence. This estimate was within the uncertainty range of the statistical analysis performed.
- Frequency of consumption: at 1.5 and 2 times a week for horse meat, and 2 and 4 times a week for beef-based products, which corresponded to the highest average and 95th percentile frequency of consumption estimated among consumers only across the surveys with at least 7 days of consumption available in the Comprehensive database.
- Prevalence of horse meat in beef products: 0.1 %, 1 % corresponding to the results of the industry tests published UK-FSA (UK-FSA, 2013a), and 2 % as a high bounded scenario, extrapolated from the results of the industry tests published by FSAI (FSAI, 2013a,b) (1.7 %) and the preliminary results of the UK-FSA UK wide survey of beef products which revealed up to 5 positive samples out of 362 (1.4 %) (UK-FSA, 2013b).

The estimated likelihood of dietary exposure to phenylbutazone is higher for the consumers of horse meat than for the consumers of beef-based products potentially adulterated with horse meat. Based on a horse meat consumption frequency of twice a week and a prevalence of phenylbutazone in horse meat at 3.5 %, a consumer would be exposed to phenylbutazone once every 4 months. Using a conservative scenario (beef-based products consumption frequency

of four times a week, 2 % of the beef-based products containing horse meat, 3.5 % (see Section 3.1) of horse meat containing phenylbutazone), a consumer of beef-based products could be exposed to phenylbutazone once every 6.8 years.

At the population level, and across different country and age groups this would represent from up to 144 to up to approximately 30,300 individuals per 100 million potentially exposed each day to phenylbutazone through the consumption of horse meat or horse meat products, and from up to 5 to up to 36,800 individuals per 100 million through the consumption of beef-based products adulterated with horse meat.

It must be taken into consideration that these estimates are based on the prevalence of fraudulent practices as observed during the first quarter of 2013, which is expected to decrease with time.

Table B5: Likelihood of dietary exposure to phenylbutazone.

	Expected frequency of exposure			
	Years ^(a)	Individuals per 100 million ^(b)	Years ^(a)	Individuals per 100 million ^(b)
	With a prevalence of phenylbutazone in horse meat of 0.13 % ^(c)		With a prevalence of phenylbutazone in horse meat of 3.5 % ^(d)	
From the consumption of horse meat				
• 1.5 times a week	9.8	5 - 844	0.4	144 – 22,727
• 2 times a week	7.4	7 – 1,126	0.3	192 – 30,303
From the consumption of beef-based products				
• 2 times a week with a prevalence of horse meat in beef-based products of:				
0.1 %	7,376	0.2 – 34	274.0	5 – 920
1.0 %	737.6	2 – 342	27.4	49 – 9,200
2.0 %	368.8	4 – 683	13.7	97 – 18,400
• 4 times a week with a prevalence of horse meat in beef-based products of:				
0.1 %	3,688	0.4 – 68	137.0	10 – 1,840
1.0 %	368.8	4 – 683	13.7	97 – 18,400
2.0 %	184.4	7 – 1,367	6.8	194 – 36,800

(a): Period of time expressed in years within which a consumer could be exposed at least once to a product containing phenylbutazone

(b): Range of individuals per 100 million is the minimum – maximum number of individuals per 100 million estimated to be exposed each day to a product containing phenylbutazone across the population groups taken into account (N = 28 for horse products and N = 76 for beef products). The population groups taken into account are described in Sections B4.1 and B4.2 of this Appendix.

(c): 0.13% is the average percentage of horse samples positive to phenylbutazone weighted according to the production level of each country estimated from the results of the National Residue Control Plan (Table B1)

(d): 3.5% is the percentage of horse sample positive to phenylbutazone found in the UK during the first months of 2013, particularly in the framework of the '100% testing of horse carcasses'.

B6 Levels of exposure to phenylbutazone

Considering the likelihood of exposure to phenylbutazone, only infrequent events of exposure are foreseen at the individual level for both horse meat consumers and for consumers of beef-based products adulterated with horse meat. For this reason, the estimation of chronic exposure levels were not considered relevant and, only the acute levels resulting from a single day of exposure were estimated. The highest portion sizes expressed relatively to the body weight observed among population groups with at least 5 consumption events for the average and 60 consumption events for the 95th percentile were retained (Tables B2 and B4). Concerning the beef based products, only the meat

burgers/balls, minced/ground beef meat and beef stewed were considered as they were associated with the highest portion sizes. It was assumed that respectively 90 %, 80 % and 30 % of the meat content of meat burgers/balls, beef stewed and minced/ground beef meat was horse meat, corresponding to the highest percentage of horse meat found in the industry tests published by the UK-FSA, FSAI or notified to the RASFF. Considering the uncertainty around the levels of phenylbutazone in horse meat, two scenarios were considered:

- A : As a high bounding case scenario, the highest level of phenylbutazone found to date in a kidney sample was considered (1,900 µg/kg), assuming that levels found in kidney could also be found in horse muscle or that meat products could also be adulterated with horse offal.
- B: In another scenario, the highest level of phenylbutazone reported to date in a muscle sample of horse was considered (19.2 µg/kg).

Table B7: Acute dietary exposure level to phenylbutazone.

	Children (up to 10 years)		Adolescents and adults (above 10 years)	
	Average	P95	Average	P95
Scenario A: residue level of 1,900 µg/kg				
From horse products consumption				
• Horse meat	7.22	-*	3.61	7.03
From beef products consumption:				
• Meat burgers/balls (90 % horse meat)	9.69	15.55	4.42	9.00
• Beef stewed (80 % horse meat)	5.92	7.30	4.27	5.38
• Minced/ground beef meat (30 % horse meat)	2.24	4.50	1.38	2.74
Scenario B: residue level of 19.2 µg/kg				
From horse products consumption				
• Horse meat	0.07	-*	0.04	0.07
From beef products consumption:				
• Meat burgers/balls (90 % horse meat)	0.10	0.16	0.04	0.09
• Beef stewed (80 % horse meat)	0.06	0.07	0.04	0.05
• Minced/ground beef meat (30 % horse meat)	0.02	0.05	0.01	0.03

P95: 95th percentile.

*: not enough data available to derive a large portion size of horse meat for children.

The acute levels of exposure were higher in children than in adolescents and in adults (Table D2). Assuming exposure via horse meat contaminated at the highest levels measured in horse samples (1,900 µg/kg phenylbutazone measured in kidney), the average acute exposure from the consumption of horse meat or adulterated beef-based products was

estimated to range across the population groups from 2.24 to 9.69 µg/kg b.w. per day for children and from 1.38 to 4.42 µg/kg b.w. per day for adolescents and adults. The 95th percentile acute exposure was estimated to range across the population groups from 4.50 to 15.55 µg/kg b.w. per day for children, and from 2.74 to 9.00 µg/kg b.w. per day in adolescents and adults. When considering horse meat containing phenylbutazone at a level equal to that measured in the only positive muscle sample reported (19.2 µg/kg), average acute exposure levels ranging from 0.02 to 0.10 µg/kg b.w. per day for the children and from 0.01 to 0.04 µg/kg b.w. per day for the adolescents and adults were estimated. The 95th percentage acute exposure levels were estimated to range from 0.05 to 0.16 µg/kg b.w. per day for the children, and from 0.03 to 0.09 µg/kg b.w. per day for the adolescents and adults.

C. Estimation of combined likelihood for an individual to be both a subject sensitive to develop aplastic anaemia and to be exposed to phenylbutazone from consumption of horse meat

Table C1: Combined likelihood was calculated considering the likelihood of dietary exposure (See Appendix B, Table B5) to be exposed and the likelihood to be a subject susceptible to aplastic anaemia (1:30000)

	Likelihood of dietary exposure	Combined likelihood
	Individuals per 100 million	
From the consumption of horse meat		
• 1.5 times a week	144 – 22,727	$4.8 \times 10^{-11} - 7.58 \times 10^{-9}$
• 2 times a week	192 – 30,303	$6.4 \times 10^{-11} - 1.01 \times 10^{-8}$
From the consumption of beef-based products		
• 2 times a week with a prevalence of horse meat in beef-based products of:		
0.1 %	5 – 920	$1.67 \times 10^{-12} - 3.07 \times 10^{-10}$
1.0 %	49 – 9,200	$1.63 \times 10^{-11} - 3.07 \times 10^{-9}$
2.0 %	97 – 18,400	$3.23 \times 10^{-11} - 6.13 \times 10^{-9}$
• 4 times a week with a prevalence of horse meat in beef-based products of:		
0.1 %	10 – 1,840	$3.33 \times 10^{-12} - 6.13 \times 10^{-10}$
1.0 %	97 – 18,400	$3.23 \times 10^{-11} - 6.13 \times 10^{-9}$
2.0 %	194 – 36,800	$6.47 \times 10^{-11} - 1.23 \times 10^{-8}$

D. Source of uncertainty

The evaluation of the inherent uncertainties in the assessment of exposure to phenylbutazone has been performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006). In addition, the report on 'Characterizing and Communicating Uncertainty in Exposure Assessment' has been considered (WHO-IPCS, 2008).

The prevalence and levels of exposure were estimated on the ground of the available datasets generated by the EU MSs in the context of the National Residue Control Plans. Those monitoring programmes are designed for enforcement purposes and not with the aim of estimating the exposure for consumers. In this respect several uncertainties were identified, namely:

- Some monitoring programmes may be designed to target suspect samples, leading to a potential overestimation of the prevalence.
- In addition, a low number of samples were taken in some countries with high production of horse meat, bringing a considerable uncertainty in the estimation of the parameters of interest.
 - o All results were taken into account to estimate the prevalence, independently from the matrices analysed. It was assumed that if phenylbutazone was found in a matrix other than muscle, then it might have been also present in muscle. Nevertheless, considering the pharmacokinetic profile of phenylbutazone in horses, the substance is expected to be most frequently found and at higher levels in kidney than in muscle. This may have led to an overestimation of the prevalence of phenylbutazone in horse meat.
 - o In addition, a low number of samples were taken in some countries with high production of horse meat, bringing a considerable uncertainty in the estimation of the parameters of interest. A quantitative uncertainty analysis was conducted through a Bayesian approach which indicated that the true prevalence of contaminated carcasses out of the total throughput at EU level would be below 7.2 % with a 95 % confidence interval (see paragraph D2). However, assuming 3.5 % of the horse meat present in the EU market being positive to phenylbutazone was considered as a high bounding scenario (see Annex B).
 - o The acute exposure was assessed based on scenarios considering the highest levels of phenylbutazone measured up-to-date. A high percentile of contamination, such as the 95th percentile, is usually preferred, as it is more stable and less biased by potential outliers. Nevertheless, for both kidney and muscle, the 95th percentile of contamination was falling into the left censored area (not detected results). This is considered as a conservative scenario.

The prevalence of fraudulent practices consisting in replacing beef meat by horse meat in beef-based products is currently unknown. Different scenarios were set, based on the results available from industry tests in UK and Ireland, and preliminary results from the UK-FSA survey, which showed between 1 and 2 % of beef-based products samples tested positive to horse meat. This is considered as a conservative estimate, as the positive samples were withdrawn from the market. Moreover, it is not known whether horse offal would also enter the food chain illicitly. An exposure scenario considering the levels of phenylbutazone found in horse kidney was considered.

Based on the highest rate of horse meat found to date in beef-based products, it was considered that 90 % of meat present in beef burgers and meat balls, 80 % of the meat present in beef stewed and 30 % of the minced/grounded beef meat corresponded to horse meat. This is considered as a conservative estimate.

The EFSA Comprehensive European Food Consumption Database does not cover all the European population (some Member States/age groups, particularly for children are missing). The consumption surveys are usually covering 1 up to 7 days of reporting, and hence they are of limited reliability for the characterisation of levels and frequency of consumption of rarely consumed foods, such as horse meat. Different frequencies of consumption were simulated. A frequency of two consumption events per week was assumed as conservative for horse meat consumers.

In the EFSA Comprehensive Database, the kind of meat present in a meat product is not systematically specified. Except for meat pie, pasta with meat, meat burger, meatballs and sauce Bolognese, only the consumption data specifically mentioning the presence of beef meat were taken into account. For meat pie, pasta with meat, meat burger, meatballs and sauce Bolognese, both the consumption data mentioning the presence of beef meat and meat of unspecified origin were taken into account. Moreover, the EFSA Comprehensive Database contains data mostly expressed at the ingredients level. For example, in some surveys, beef goulash is reported as a composite dish “meat goulash”. In that case, the consumption level includes both the meat and other ingredients entering in the composition of beef goulash. But in most surveys, the beef part of that composite dish has been reported as an ingredient “beef meat”. In that case, the consumption level refers only to the meat content. Some adjustments were made, but this adds uncertainty in the consumption estimates of beef-based products.

The exposure scenarios are based on a single “European” estimate, without considering the possible variability throughout Europe. As a consequence, the exposure estimates (both likelihood and level) produced at a population group level may sometimes be overestimated, sometimes underestimated. The data available don't allow to describe the variability of occurrence. Indeed, as the kind of matrices analysed differs according to the country, it is not possible to distinguish a matrices effect from a country effect. Moreover, the results taken into consideration mostly correspond to samples taken in slaughterhouses. In the framework of a global European market, this may not reflect the occurrence levels in meat present on the each country's market.

The exposure scenarios are based on the presence of phenylbutazone in horse meat consumed as such, or illicitly present in beef-based products. It must be underlined that phenylbutazone has been reported in bovine animals (EFSA, 2011a, 2012, 2013). This source of exposure has not been taken into account as such, leading to a potential underestimation of the dietary exposure levels of the European population to this substance.

Because of the lack of data on oxyphenbutazone and on its concurrent presence with phenylbutazone, this metabolite has not been taken into consideration in the exposure assessment, which may lead to an underestimation of the risk.

Finally, no information is available on the incidence of aplastic anaemia related to the exposure to phenylbutazone at doses in the range of those estimated to occur from the consumption of horse meat (approximately 15 µg/kg b.w. per day or below). The incidence of 1 case of aplastic anaemia over 30000, observed in the therapeutic use of the substance at doses typically ranging from 200 to 400 mg/person per day, was considered for the calculation of the combined likelihood that a subject is both sensitive to develop aplastic anaemia and is exposure to phenylbutazone from the consumption of horse meat, likely leading to an overestimation of the risk.

D.1 QUANTITATIVE ANALYSIS AROUND THE PREVALENCE OF PHENYLBUTAZONE IN HORSE CARCASSES

A stochastic approach based on binomial process and Bayesian inference principles was applied in order to include uncertainty and variability around the parameter of interest. In the specific case, the estimation of the prevalence was performed by means of a binomial process. The true underlying prevalence was estimated considering the data reported in Table B1 and assuming a Beta probabilistic distribution with an uninformative prior (considering the number of samples to be not representative of the overall target population). Under this approach the prevalence of horse carcasses containing phenylbutazone can be expressed as follows:

$$p \sim \text{Beta}(x, s + 1, n - s + 1) = \frac{x^s(1-x)^{n-s}}{\int_0^1 t^s(1-t)^{n-s} dt} \quad (1)$$

Where **n** is the number of tested samples and **s** is the number of positive samples.

Once the underlying prevalence and its uncertainty were calculated, it was possible to estimate, for each country, the actual number of positive carcasses out of the total throughput. The overall prevalence, i.e. the prevalence of positive carcasses at EU level, could then be estimated as follows:

$$p_{EU} = \frac{\sum_{i=1}^m \text{Cases}_i}{\sum_{i=1}^m N_i} \quad (2)$$

where **m** is the number of reporting countries, **Cases** is the estimated number of real cases for each country and **N** is the average annual throughput per country.

Data were stored and managed in Microsoft Excel. The modelling exercise and the simulations were performed using the software @Risk⁶. The simulation ran 10.000 iterations.

Results

Equation (1) was used to estimate the underlying true prevalence at country level. The values of the 95th percentile confidence level and the mode are reported in Table D1.

Table D1: Data on sample size (n) and number of positive tests (s) for each country. The mode represents the most likely value estimated for the prevalence. 95 % represents the 95th percentile of the uncertainty distribution around the prevalence at country level.

Member State	s	n	Prevalence	
			Mode	95 %
Belgium	0	132	3.77E-05	0.0223
Bulgaria	0	8	5.57E-04	0.2830
Czech Republic	1	24	4.01E-02	0.1761

⁶ @RISK, Risk Analysis Add-In for Microsoft Excel, Version 5.0.1: Professional Edition; Copyright 2008, Palisade Corporation

Denmark	1	33	3.18E-02	0.1321
Finland	0	30	1.62E-04	0.0921
France	0	329	1.52E-05	0.0090
Germany	1	188	5.59E-03	0.0249
Hungary	0	3	1.25E-03	0.5270
Ireland	1	361	2.77E-03	0.0130
Italy	0	47	1.04E-04	0.0605
Latvia	0	7	6.27E-04	0.3122
The Netherlands	0	36	1.36E-04	0.0777
Poland	0	92	5.39E-05	0.0317
Portugal	0	9	5.01E-04	0.2588
Romania	0	2	1.67E-03	0.6315
Slovenia	0	6	7.17E-04	0.3482
Spain	0	58	8.51E-05	0.0495
Sweden	1	225	4.31E-03	0.0208
United Kingdom	32	796	4.00E-02	0.0536
SUM	37	2 386		

As can be seen from Table D1, the uncertainty around the prevalence at country level is directly related to the number of collected samples.

The underlying prevalence at European level was then estimated using Equation (2).

The result from a simulation (10,000 iterations) gives the cumulative distribution shown in Figure D1, which allows estimating **with a 95 % confidence that the prevalence of horse carcasses containing phenylbutazone at EU level is below 7.2 %**.

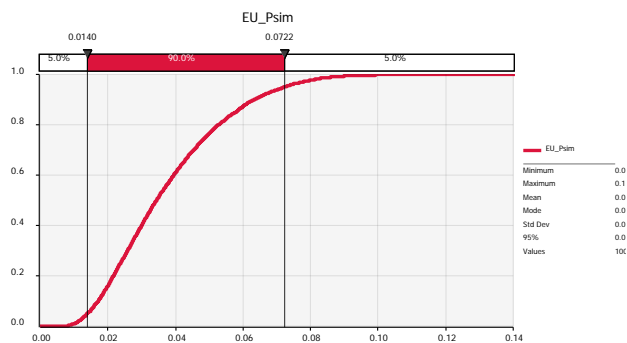


Figure D1: Estimated prevalence of horse carcasses containing phenylbutazone at European Union level. It can be stated with a 95 % confidence that the true underlying prevalence is below 7.2 %.

Abbreviations

ADI	Acceptable daily intake
b.w.	Body weight
Comprehensive Database	EFSA Comprehensive European Food Consumption Database
CHMP	EMA Committee for Medicinal Products for Human Use
CVMP	EMA Committee for Medicinal Products for Veterinary Use
EFSA	European Food Safety Authority
ELISA	Enzyme-Linked Immunosorbent Assay
EMA	European Medicines Agency
EU	European Union
FCI	Food Chain Information
FSAI	Food Safety Authority of Ireland
HPLC-DAD	High performance liquid chromatography – diode-array detection
HPLC-MS	High performance liquid chromatography – mass spectrometry
LC-MS/MS	Liquid chromatography – tandem mass spectrometry
LOQ	Limit of quantification
MRL	Maximum residue limits
MS	Member State
NOEL	No-observed-effect level
NRCP	National Residue Control Plan
NSAID	Non-steroidal anti-inflammatory drug
PCR	Polymerase chain reaction
RASFF	Rapid Alert System for Food and Feed
UK FSA	United Kingdom Food Standards Agency