The European Agency for the Evaluation of Medicinal Products

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

PARACETAMOL

SUMMARY REPORT

1. Paracetamol (synonym: acetaminophen) is an antipyretic and analgesic substance of the para-aminophenol group of the non-steroidal anti-inflammatory drugs. It is used in pigs as a 10% medicated premix at a dosage rate of 15 to 30 mg/kg bw/day for 3 to 5 consecutive days in the treatment of painful disease states associated with pyrexia. In some Member States, oral powder containing paracetamol is also used in cattle for fermentation disorders and acetonemia and poultry for treatment of painful diseases and pyrexia. No further information concerning the dosage and duration of treatment in these species was available.

   Paracetamol is used in human medicine for mild to moderate pain and pyrexia and is available in a wide range of proprietary over-the-counter preparations. The usual adult oral dose is 0.5 to 1 g (approximately 8.3 to 16.7 mg/kg bw) every 4 to 6 hours up to a maximum of 4 g (approximately 66.7 mg/kg bw) daily.

2. The analgesic properties of paracetamol are primarily due to centrally mediated cyclo-oxygenase inhibition by reversible, non-competitive binding, preventing the conversion of arachidonic acid to prostaglandin metabolites. It is only weakly effective at peripheral sites, possibly due to the high concentrations of peroxides found in inflammatory lesions and therefore has only a minor role in reducing pain through the protection of receptors from the effects of pain mediators.

   In a study of pediatric dosing regimens, 4-hourly doses of 10 to 15 mg/kg bw were necessary to achieve optimal reduction of pyrexia while oral doses of 5 mg/kg bw were inadequate. Another study concluded that a pediatric dose of 5 mg/kg bw was no more effective as an antipyretic than the placebo. It was concluded that the pharmacological LOEL in human infants was 5 mg/kg bw.

3. After oral administration paracetamol is rapidly and almost completely absorbed in humans and animal species. Virtually no absorption occurs from the stomach. Absorption from the small intestine is rapid, the estimated $t_{1/2}$ for absorption is about 6.8 minutes in humans. Plasma elimination $t_{1/2}$ is about 1 to 2.5 hours after a therapeutic dose in healthy adults.

   Distribution is rapid and uniform. The compound is weakly lipid soluble and appears in most body fluids. The apparent volume of distribution is about 1 l/kg. Plasma binding is low in humans at therapeutic levels, increasing at higher doses. Bioavailability in humans ranges from 68% at a dose of 0.5 g to 90% at a dose of 1 to 2 g. In dogs, an oral dose of paracetamol was reported to be distributed in almost all organs, except fat. Highest tissue levels were found in liver and kidneys.

4. In humans and animals paracetamol is metabolised in the liver by multiple pathways, primarily to glucuronide (approximately 60%) and sulphate conjugates (approximately 35%). A third pathway for hepatic metabolism involves cytochrome P_{450}-catalysed formation of *N*-acetyl-*p*-benzoquinoneimine, a reactive intermediate that may be reduced back to paracetamol in an NADPH-dependent reaction or detoxified to a glutathione conjugate. In overdose, toxicity is probably due to depletion of hepatic glutathione and *N*-acetyl-*p*-benzoquinoneimine reacting with sulphydryl groups in hepatic proteins. Conjugation with sulphate and glucuronic acid follow Michaelis-Menton kinetics; formation of the sulphate is especially prone to saturation (occurring

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in adults with as little as 2 g of paracetamol. Increases in paracetamol intake could exceed the capacity of the saturable pathways, increasing formation of the reactive metabolite and increased levels of cysteine and mercapturic acid metabolites. Stores of available glutathione in the liver are dependent upon nutritional status. Low intake of protein and sulphur-containing amino acids could result in decreased metabolic capacity.

No laboratory species provides an ideal animal model for human metabolism, but the hamster was closest with respect to metabolites formed and susceptibility to acute toxic effects. No repeated dose, reproduction or long-term toxicity studies were available in this species. The rat is different from humans and other species. The major urinary metabolite is the sulphate, rather than the glucuronide and is reported to be more resistant to liver necrosis than hamsters and mice. Rats converted only 3 to 4% of non-toxic doses to the mercapturic acid (an index of the toxic *N*-acetyl-*p*-benzoquinoneimine intermediate), compared to 13 to 15% in mice and hamsters. In the dog, the glucuronide was the major urinary metabolite, in the cat the sulphate was predominant. It is, therefore, probable that the mouse is the best toxicological model from those species.

The urinary route is the primary route of excretion of paracetamol and its conjugates, with 85 to 95% of an ingested dose appearing in the urine within 24 hours, although less than 5% of the dose is excreted as parent compound. Large amounts of the glucuronide and glutathione metabolites are initially excreted in the bile. The excreted glucuronide is hydrolysed to the parent compound, which is reabsorbed, and the glutathione conjugate is converted to the 3-cysteinyl conjugate by γ-glutamyl-transpeptidase. At therapeutic doses 45 to 55% of the total oral dose was excreted by human volunteers as glucuronide, 20 to 30% as sulphate and 15 to 25% as mercapturic acid. As the dose approached 4 g, the amount of sulphate produced reached a plateau, but levels of glucuronide, cysteine and mercapturic acid metabolites increased. At around 10 g, levels of glucuronide were similar, the sulphate fraction dropped to 11% and cysteine and mercapturic acid conjugates rose to 37%. Studies of urinary metabolites in humans show 85 to 100% of the dose was excreted in the first 24 hours. Changes in the relative amounts of the various metabolites correlated with the degree of liver damage, with moderate to severe damage occurring in individual with more than 30% cysteine and mercapturic acid metabolites and less than 60% as glucuronide. The toxicity of paracetamol appears to be mainly dependent on ability to produce glutathione conjugates and the degree of hepatic glutathione depletion. Rates of production and depletion are dependent on concentrations of paracetamol and glutathione.

Extrahepatic metabolism of paracetamol and its metabolites has been reported. The kidney is a major site for the conversion of the 3-glutathionyl conjugate to the 3-cysteinyl conjugate. This reaction also occurs in the intestinal wall, which is rich in γ-glutamyl-transpeptidase. Conversion of paracetamol to *p*-aminophenol also occurs primarily in the kidney. In patients with impaired renal function conjugated metabolites, but not parent drug, accumulate in the blood. Plasma half-life was greatly increased in patients with liver damage, excretion of conjugated paracetamol was slower and less of the dose was conjugated.

5. Excessive single oral doses of paracetamol cause hepatotoxicity and nephrotoxicity in laboratory animals. Oral LD_{50} values are around 400 to 900 mg/kg bw in the mouse and more than 2000 mg/kg bw in the rat, rabbit and guinea pig. Lethal oral doses in the dog and cat are around 500 mg/kg bw and more than 50 mg/kg bw, respectively. In the latter two species methaemoglobinaemia is the predominant effect, generally not seen in other species but reported in overdosed children. Bone marrow toxicity has been reported in an *in vivo* micronucleus test in the rat using high oral doses (three times 500 mg/kg bw at a 4-hour interval or once 1500 mg/kg bw).

6. Dietary doses of up to 1000 mg/kg bw for 14 days are well tolerated in the mouse. In the rat, decreased bodyweight gain was reported at doses higher than 800 mg/kg bw.

7. In the mouse, after dietary exposure to paracetamol for 13 weeks, at doses of 200, 400, 800, 1600, 3200 and 6400 mg/kg bw/day hepatotoxicity, organ weight changes and deaths were observed. The NOEL was 400 mg/kg bw/day in males and 800 mg/kg bw/day in females.

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In rats, exposed to dietary doses of 100, 200, 400, 1600 or 3200 mg/kg bw/day for 13 weeks, deaths occurred at the highest dose. There was evidence of renal toxicity in males of the 1600 mg/kg bw/day dose group and hepatic and renal toxicity in both sexes at 3200 mg/kg bw/day. Treatment related increases in liver and kidney weights were seen at all doses. No NOEL could be identified in rats.

8. Hepatic necrosis is believed to be due to a reactive metabolite(s) formed during the cytochrome P_{450}-catalysed metabolism. Hepatotoxic doses of labelled paracetamol in mice resulted in covalent binding of labelled material to liver protein in levels that paralleled the degree of severity of the hepatic necrosis. It is postulated that paracetamol was *N*-oxidised to *N*-hydroxyacetaminophen, then dehydrated to the arylating agent *N*-acetyl-*p*-benzoquinoneimine. *In vitro* studies in isolated rat hepatocytes suggest that the cytotoxic effects of paracetamol and *N*-acetyl-*p*-benzoquinoneimine are due to disruption of Ca^{2+} homeostasis. However, two studies have shown that in individual cells treated with paracetamol, the increase in cytosolic Ca^{2+} accompanies, rather than precedes, loss of viability.

   It is suggested that under normal circumstances, *N*-acetyl-*p*-benzoquinoneimine is detoxified by glutathione, possibly by a glutathione-*S*-transferase in the liver cytosol. As the paracetamol dosage increases, glutathione stores are depleted and glucuronidation and sulfatation pathways become saturated allowing *N*-acetyl-*p*-benzoquinoneimine to covalently bind to cell macromolecules. Significant hepatic necrosis was not reported until hepatic glutathione levels were depleted to 20 to 30% of normal. *N*-acetyl-*p*-benzoquinoneimine is also easily reduced by ascorbic acid.

   Lipid peroxidation has also been reported at rapid rates in liver homogenates from paracetamol-treated rats and mice and *in vitro* in paracetamol-treated rat and mouse hepatocytes. It also does not occur until glutathione levels were depleted to 20 to 25% of normal. The role of lipid peroxidation in paracetamol toxicity is controversial, evidence against an *in vivo* role in paracetamol hepatotoxicity includes the late appearance of products of lipid peroxidation and lack of effect of agents that protect against lipid peroxidation and hepatic damage caused by other compounds, e.g. carbon tetrachloride. An alternative suggestion of paracetamol hepatotoxicity is the conversion to a semiquinone free radical and/or an active oxygen species, e.g. hydrogen peroxide or oxygen radical.

   Renal cortical necrosis due to acute high-dose exposure and chronic renal disease following long-term usage or exposure occurs in humans and animals. In mice, the events leading to renal tubular necrosis appear similar to those in the liver and may involve cytochrome P_{450}-catalysed formation of a reactive intermediate that depletes glutathione arylates macromolecules and is consistent with the cortical location of cytochrome P_{450} mixed-function oxidase activity. Studies in rats suggest that the early stage of paracetamol nephrotoxicity might be due to renal haemodynamic changes (impaired glomerular filtration rate and *p*-aminohippuric acid clearance), leading to an alteration in tubular function principally in the distal structures in the medulla. It was also noted that renal damage was observed both in the presence and absence of hepatic damage. Studies with isolated rabbit renal medulla microsomes also indicate that paracetamol may be activated via co-oxygenation during prostaglandin biosynthesis by peroxidase activity of medullary prostaglandin synthetase.

9. In a tolerance study pigs received a single oral bolus of 10% dietary premix, corresponding to 0, 2.5, 5 or 10 times the recommended therapeutic dose. No adverse clinical effects were seen in any groups. In the blood biochemistry results, significant changes from pre-treatment values were seen for some parameters; increased glucose in the controls; increased urea and decreased creatinine at 50 mg/kg bw; decreased creatinine, serum alanine aminotransferase and lactate dehydrogenase at 100 mg/kg bw; and increased glucose at 200 mg/kg bw. No treatment-related trends were apparent, apart from raised glucose levels in all groups and decreased creatinine in all treated groups (raised in controls).

10. Data on reproductive toxicity are limited and the studies that have been conducted are not in accordance with current standards. In a 5-generation feeding study using ABC-A mice parental and foetal survival and reproductive performance were significantly reduced at all dose levels (130 to 1210 mg/kg bw).

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In a reproductive toxicity study, Swiss CD1 mice received paracetamol at doses of 357, 715, 1430 mg/kg bw. Reductions in fertility and neonatal survival were seen in the F0 generation and decreases in F1 pup weights at 1430 mg/kg bw. The NOEL for these effects was 715 mg/kg bw. Sperm abnormalities were also reported at this dose-level, although this was not investigated in the lower dose groups (357 and 715 mg/kg bw). Hence, no overall NOEL can be identified.

11. In an embryotoxicity study in the rat, pregnant dams were exposed to 0, 125 or 250 mg/kg bw paracetamol on gestation days 8 to 19. Resorptions were seen only at 125 mg/kg bw accompanied by a significant decrease in foetal length. No NOEL could be identified due to the effects observed at the lowest dose and the limitations of the study, which was not in accordance with current standards.

12. Neither paracetamol nor its metabolite *N*-acetyl-*p*-benzoquinoneimine caused gene mutations in bacteria in a series of *Salmonella*-microsomal assay. Covalent binding to DNA has been observed *in vitro* and *in vivo*. The results of assays for DNA repair were inconsistent. Paracetamol did not affect microtubule polymerisation but did cause sister chromatid exchanges *in vitro* and clastogenicity both *in vitro* and *in vivo*. The causal mechanisms of these effects have not been fully elucidated, but may involve similar mechanisms to those causing hepatotoxicity and nephrotoxicity via reactive intermediate metabolites. They appear to only occur at cytotoxic concentrations of 3 to 10 times the plasma concentration associated with therapeutic usage, indicating that there would be a threshold dose below which no mutagenicity would be seen and that this threshold is above the therapeutic dose range. Some studies have shown evidence of clastogenicity *in vivo* in humans. However, the methodology and interpretation of these studies have been criticised and a recent well-controlled, double-blind study a using larger numbers of subjects found no evidence of clastogenic effects. Reliable studies on the effects of paracetamol on germ cell DNA are not available, but there is no reason to suspect that these would occur except at high cytotoxic doses. Overall, it can be concluded that paracetamol does not cause gene mutations. However, there was evidence of chromosomal damage at high doses in experimental studies. Studies for clastogenic effects in humans were equivocal, the most recent study being negative.

13. Neoplastic effects (liver tumours in mice and bladder and liver neoplasia in rats) were only seen in older studies at very high doses and several negative studies using equally high doses have been reported. Recent studies conducted in the framework of the National Toxicology Program of the U.S.A. in the rat and mouse found no evidence of carcinogenicity. An increase of mononuclear cellular leukaemia (large granular lymphocyte lymphoma) was seen in female rats. However, this was not accompanied by increased mortality and incidence was decreased in treated male animals. Increased severity of nephropathy was seen in the male animals at all doses. In mice NOELs (thyroid follicular hyperplasia) were 90 and 600 mg/kg bw/day for males and females, respectively. There is no evidence to indicate that low doses of paracetamol (such as those, which may occur as food residues) have any associated cancer risk.

14. Paracetamol is not reported to have any immuno-modulating properties, although its anti-inflammatory action at high doses includes suppression of antigen-antibody reactions.

15. No data were provided on the microbiological properties of the substance however these data were not considered necessary for this class of compound.

16. Maximum recommended human therapeutic doses range from 50 to 67 mg/kg bw/day in adults and 20 to 60 mg/kg bw/day in children. Lethal doses in humans are in the region of 250 to 500 mg/kg bw. Acute effects in humans after overdose are principally centrilobular hepatic necrosis and renal tubular necrosis. Chronic exposure to therapeutic doses may be associated with chronic renal disease, a positive association with cancer of the ureter was reported in one study, but not in others. Epidemiological studies of chronic paracetamol usage are complicated by exposure to other analgesics and other drugs. No significant associations with congenital malformations through use in pregnancy have been reported. Methaemoglobinaemia has been reported in overdosed children. Paracetamol is indicated for use during pregnancy.

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17. Based on the pharmacological LOEL of 5 mg/kg bw in human infants, an ADI of 0.05 mg/kg bw was calculated by applying a safety factor of 100. The safety factor of 100 was considered justified because the LOEL in human infants was very close to the lowest therapeutic dose for adults. No toxicological ADI can be derived because no NOELs could be identified for chronic renal and hepatic effects in the rat and reproductive effects in the mouse. The lowest doses used in the toxicity studies in rats and reproductive studies in the mouse were higher than the only NOEL identified in the toxicity studies for thyroid hyperplasia in a 2-year chronic mouse study (90 mg/kg bw), and these effects were only observed at doses several orders of magnitude greater than the pharmacological LOEL. Furthermore, it has been established that these effects are due to the reactive intermediates that are formed at extremely high doses at which the glucuronidation capacity of the liver is overwhelmed.

18. After oral (in feed) administration of 15 mg/kg bw paracetamol to male and female Landrace pigs, a C_{max} value of 3560 µg/l was observed around 2 hours after treatment. The AUC_{0-24} was 24.4 µg.h/l and the bioavailability was estimated to be 74% [F ($AUC_{diet}/AUC_{intravenous}$)]. Bioavailability was slightly higher [F ($AUC_{bolus}/AUC_{intravenous}$) equals 77%)] when the dose was given as an oral bolus. After repeated administration in the feed, mean plasma concentrations were 440, 530, 480 and 340 µg/l on the second, third, fourth and fifth days of treatment respectively, indicating no accumulation. During the course of this study, the binding of paracetamol to plasma proteins was shown to be 11.2 to 21.6% for plasma concentrations in the range 100 to 300 µg/l.

19. In a GLP-compliant residue depletion study, Large White Landrace – Pietrain pigs were fed diets containing ^{14}C-paracetamol, at a dose equivalent to 30 mg/kg bw/day for 5 days. Groups of 2 males and 2 females were slaughtered at 12, 24 or 72 hours after the end of treatment. Total residues in tissues were determined using liquid scintillation counting. Mean residues in liver depleted from 14 100 µg equivalents/kg at 12 hours to 10 900 µg equivalents/kg at 24 hours and 6710 µg equivalents/kg at 72 hours. Mean residues in kidney depleted from 7450 µg equivalents/kg at 12 hours to 2250 µg equivalents/kg at 24 hours and 1190 µg equivalents/kg at 72 hours. Mean residues in skin with fat depleted from 1280 µg equivalents/kg at 12 hours to 750 µg equivalents/kg at 24 hours and 540 µg equivalents/kg at 72 hours. Mean residues in muscle were 660 µg equivalents/kg at 12 hours after the end of treatment and were below the limit of quantification of the assay (100 µg equivalents/kg) in most samples taken at later time points.

    The tissue samples were subsequently analysed for residues of paracetamol using an analytical method based on HPLC. Mean residues of paracetamol in liver were 522 µg/kg 12 hours after the end of treatment and were below the limit of quantification (100 µg/kg) in 2 out of the 4 samples taken 24 hours after treatment. Mean residues of paracetamol in kidney were 498 µg/kg 12 hours after the end of treatment and were below the limit of quantification (100 µg/kg) in 3 out of the 4 samples taken 24 hours after treatment. Residues in skin with fat 12 hours after the end of treatment were in the range from below the limit of quantification to 334 µg/kg and were below the limit of quantification in 3 out of the 4 samples taken 24 hours after the end of treatment. Residues of paracetamol in all muscle samples were below the limit of quantification. This study showed that 12 hours after the end of treatment, approximately 4% of the total residues in liver, 7% of the total residues in kidney and 17% of the total residues in skin + fat were present as paracetamol.

    Due to the very transitory nature of the metabolites *p*-aminophenol and *N*-acetyl-*p*-benzoquinoneimine in porcine tissues the attempts to determine residues were unsuccessful.

20. In a previous residue depletion study, Landrace pigs were given paracetamol in the feed at a concentration equivalent to 30 mg/kg bw/day for 5 consecutive days. Three pigs were killed at intervals of 1 day, 3 days, 5 days and 7 days after the end of treatment. Residues of paracetamol in tissues were determined using HPLC with UV detection. In this study, the limits of detection were 100 µg/kg for tissues and 50 µg/l for plasma. Mean residues 1 day after the end of treatment were 320 µg/l in plasma, 360 µg/kg in muscle, 290 µg/kg in liver, 180 µg/kg in kidney and 110 µg/kg in fat. At later time points, residues in all tissues were below 100 µg/kg and residues in plasma were below 50 µg/l.

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21. No residue depletion studies were provided for cattle and poultry.

22. An analytical method for the determination of paracetamol in tissues based on HPLC with UV detection is available. Validation data were provided for edible porcine tissues. Specificity was satisfactory and residues of the metabolites *p*-aminophenol and *N*-acetyl-*p*-benzoquinoneimine did not interfere in the assay. The limit of quantification was 100 µg/kg for muscle, liver, kidney and fat. The limit of detection was 50 µg/kg for muscle, liver and kidney and 20 µg/kg for fat. The method was described in the ISO 78/2 format.

Conclusions and recommendation

Having considered that:

- a pharmacological ADI of 0.05 mg/kg bw was established for paracetamol,
- although no overall toxicological ADI was established, this was considered unnecessary as the effects observed occurred only at doses several orders of magnitude higher than the pharmacological ADI and were due to reactive metabolites formed after saturation of the normal metabolic pathway,
- paracetamol is rapidly absorbed and excreted in the urine after oral administration to the target species,
- based on the mean total residues at 24 hours after the end of treatment, the consumer intake of total residues from pig tissues represents approximately 42% of the ADI;

the Committee for Veterinary Medicinal Products concludes that there is no need to establish an MRL for paracetamol and recommends its inclusion in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Paracetamol	Porcine	For oral use only

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