

## COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

### Review of potential risks from mycotoxins in the diet of infants aged 0 to 12 months and children aged 1 to 5 years

#### Cyclopiazonic acid (CPA)

##### **Background**

1. The Scientific Advisory Committee on Nutrition (SACN) is undertaking a review of scientific evidence that will inform the Government's dietary recommendations for infants and young children. The SACN is examining the nutritional basis of the advice. The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) was asked to review the risks of toxicity from chemicals in the diet of infants, which has been completed, and young children. The reviews will identify new evidence that has emerged since the Government's recommendations were formulated and will appraise that evidence to determine whether the advice should be revised. The recommendations cover diet from birth to age five years.
2. The Food Standards Agency (FSA) has completed a survey of 36 mycotoxins in the 2014 Total Diet Study (TDS) – mycotoxins analysis (FSA, to be published). The results of the survey provide information on the concentrations of aflatoxins, ochratoxin A, zearalenone, fumonisins, 3-acetyldeoxynalolenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), deoxynivalenol (DON), diacetoxyscirpenol, fusarenon-X (Fus-X), T-2 toxin, HT-2 toxin, neosolaniol, nivalenol (NIV), sterigmatocystin, citrinin, cyclopiazonic acid, moniliformin, patulin and ergot alkaloids (ergocornine, ergocorninine, ergocristine, ergocristinine, ergocryptine, ergocryptinine, ergometrine, ergometrinine, ergosine, ergosinine, ergotamine, ergotaminine) in relevant foods. Estimates of dietary exposures have been calculated for each mycotoxin for UK infants and young children aged 4 to 60 months using food consumption data taken from the Diet and Nutrition Survey of Infants and Young Children (DNSIYC) and the National Diet and Nutrition Survey (NDNS) (Bates *et al.*, 2014 & 2016; Lennox *et al.* 2013).
3. A scoping paper (TOX/2015/32) "COT contribution to SACN review of complementary and young child feeding; proposed scope of work for 1-5 years old children" was reviewed by the COT in 2015. A further scoping paper for mycotoxins was presented to the COT in 2017. This discussion paper will provide a review of the available literature for CPA.
4. CPA is produced by several species of *Aspergillus* and *Penicillium*, and is widespread in naturally contaminated agricultural raw materials. CPA is normally formed under storage conditions and may be found alongside aflatoxin in the food and feed chain. CPA has been found in a range of food types including seeds,

grains, cheeses, meat products, eggs and cow's milk (Burdock and Flamm, 2000; Chang, Ehrlich and Fujii, 2009).

5. After ingesting CPA-contaminated feeds, test animals display gastrointestinal (GI) and neurological effects. Organs affected include the liver, kidney, heart, and digestive tract, which show degenerative changes and necrosis (Ostry *et al.* 2018). Brief summaries of published studies relating to the absorption, distribution, metabolism and excretion (ADME) and toxicity of CPA are provided in the relevant sections below.

6. There is little evidence available for human toxicity due to consumption of food contaminated with CPA. Rao and Husain (1985) reported the isolation of CPA from two batches of kodo millet (*Paspalum scrobiculatum*) grain associated with incidents of 'kodua poisoning' in humans and cattle in India. It was demonstrated that strains of *A. flavus* and *A. tamarii* detected in this contaminated millet produced CPA, however the concentration of CPA was not determined in the contaminated millet.

### **Previous risk assessments**

7. Due to the limited availability of relevant toxicity data, there are currently no risk assessments or evaluations of CPA performed by European or International agencies or committees such as the European Food Safety Agency (EFSA), the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and the International Agency for Research on Cancer (IARC).

8. Risk assessments on CPA have, however, been carried out (Burdock and Flamm, 2000; De Waal, 2002; and Ostry *et al.*, 2018) and are described in more detail in the paragraphs below.

9. Lomax *et al.* (1984) performed a repeat dose toxicity study in crossbred pigs administered CPA in gelatin capsules at oral dose levels of 0, 0.1, 1.0 or 10 milli-grams per kilogram of body-weight per day (mg/kg bw/day) over 14 consecutive days. The authors reported a no observed effect level (NOEL) of between 0.01 and 0.1 mg/kg bw/day because lesions were observed in the GI tract at dose levels as low as 0.1 mg/kg bw/day.

10. In their literature review, Burdock and Flamm (2000) noted that: "The actual NOEL is likely higher than that offered by Lomax *et al.* (1984) because (1) the only gross lesion in the 1.0 mg/kg group was seen in a single animal (one of eight) in two different trials (four pigs per group in each trial), (2) the microscopic pathology was found in only two of eight pigs at the 1.0 mg/kg level and in two of eight pigs at the 0.1 mg/kg dose level, and (3) the anatomy of the porcine digestive tract in the area of the observations would tend to predispose to such a diagnosis. Therefore, although CPA may have been contributory to the gross and microscopic pathology in the high-dose animals (10 mg/kg/day), the investigators may have overestimated the effects at the lower dose levels. Based on these observations, we would estimate the NOEL in this study to be 1.0 mg/kg/day." Burdock and Flamm (2000) subsequently proposed an acceptable daily intake (ADI) of 10 µg/kg bw for CPA through the application of an uncertainty factor of 100 to the estimated NOEL of 1.0 mg/kg bw/day.

11. De Waal (2002) responded to the approach of Burdock and Flamm (2000) and argued that an ADI should safeguard life-time exposure, thus the underlying NOEL should be based on toxicity studies of prolonged duration. In this respect, De Wall considered a 90-day dog study (Nuehring *et al.* 1985) to be more appropriate than the 14-day pig study (Lomax *et al.* 1984) for the establishment of a tolerable daily intake (TDI).
12. De Waal (2002) proposed a TDI of 0.1 micro-grams per kilo-gram of body-weight per day ( $\mu\text{g/kg bw/day}$ ) CPA, based on a NOEL of 0.1 mg/kg bw/day derived from a 90-day dog study performed by Nuehring *et al.* (1985). An uncertainty factor of 1000 was applied based on uncertainties in the extrapolation from experimental animals to humans, and intraspecies variability and an incomplete database. Dose-dependent signs of vascular toxicity (mainly in the GI tract, liver and kidneys) was the critical effect observed at the 0.5 and 2.0 mg/kg bw/day dose levels.
13. In a recent literature review by Ostry *et al.* (2018), it was concluded that the data from relevant sub-chronic studies on CPA in experimental animals are inadequate to determine a TDI. In addition, results from *in vitro* genotoxicity assays are inconclusive and chronic toxicity studies are currently absent, thus it is difficult to conclude on the carcinogenicity of CPA.

## **ADME and Toxicity**

### **ADME**

14. In a study by Byrem *et al.* (1999), a single bolus injection of 20 mg CPA (in 2 millilitre (ml) 1 normal (N) NaOH) was administered to each of 4 pigs. Blood samples for CPA analysis were withdrawn at intervals between 2 minutes and 96 hours. Plasma was retained for analysis. In addition, 3 pigs ( $97 \pm 7$  kg) were provided with a diet containing 10 mg CPA/kg feed (calculated as 0.3 mg CPA/kg bw/day) for 6 days *ad libitum*. The daily feed intake was  $2.95 \text{ kg} \pm 0.23 \text{ kg}$  and plasma samples were taken on day 3, 4, 5 and 6 and skeletal muscle samples were taken within 10 minutes of exsanguination on day 6. The plasma kinetics for CPA were best described using a 3-compartment model, a rapid distribution and a large volume of distribution (49 L) in pigs given a 20 mg intra-venous (i.v.) bolus. CPA was eliminated with a half-life of 24 h. Steady-state plasma CPA levels were reached within 6 days in pigs consuming a diet containing 10 mg/kg CPA (0.3 mg CPA/kg bw/day). The measured concentrations of CPA in plasma were  $410 \pm 44$  nano-grams per milli-litre (ng/mL) and in skeletal muscle were  $469 \pm 86$  ng/g (Byrem *et al.*, 1999).
15. [ $^{14}\text{C}$ ] radiolabelled CPA was administered to Sprague-Dawley (SD) rats intragastrically (5 mg/kg, 0.6 micro-curie per kilogram ( $\mu\text{Ci/kg}$ )) and parenterally (1 mg/kg, or 0.12  $\mu\text{Ci/kg}$ ) (Norred, 1990) or orally (5 mg/kg, 0.6  $\mu\text{Ci/kg}$ ) (Norred *et al.*, 1985). The biological half-life of [ $^{14}\text{C}$ ] was 33 - 43 hours, depending on the route of administration. Radioactivity was not excreted into expired  $\text{CO}_2$  indicating that extensive metabolic degradation of CPA did not happen. CPA was readily absorbed from the GI tract into the bloodstream (maximum levels of 10 %

reached at 6 hours). The liver, heart, kidney and lung were relatively highly labelled however, approximately 45 – 50 % of the CPA dose was distributed to the muscles within the first 12 hours after dosing (Norred 1990, Norred *et al.*, 1985). CPA or its metabolites appeared in both urine and faeces, which are the major routes of excretion.

16. Chickens were dosed with 0, 0.5, 5.0 or 10 mg/kg bw CPA by crop intubation. The highest levels of CPA were found in meat 3 hours after dosing and were dose-dependent. In birds given low or mid doses, the muscle CPA content decreased rapidly. After 24 hours none and 25 % (compared to 3 hour levels) was detected, respectively. Birds given 10 mg/kg eliminated CPA from muscle at a much slower rate, with an approximate half-life of 60 hours (Norred, 1990).

#### *Acute toxicity*

17. In a study by Purchase (1971) intra-peritoneal (i.p.) injection of CPA to male Wistar-derived rats (8 - 25 mg/kg) produced hyperesthesia and convulsions followed by death in about 2 hours. Rats receiving 2.5 and 4.5 mg/kg died 1 – 3 days after dosing and rats receiving lower than 2.5 mg/kg (lowest dose 0.8 mg/kg) recovered and survived until day 10. A lethal dose 50 (LD<sub>50</sub>) of 2.3 mg/kg was calculated. Oral administration (30, 36.7, 45 and 55 mg/kg in males and 30, 36.7, 45, 55, 67.5 and 82.6 mg/kg in females) resulted in the death of 12/30 female rats within 36 hours of dosing and 3/20 and 8/20 male rats at 48 hours and between 4 and 6 days after dosing, respectively. LD<sub>50</sub>'s of 36 mg/kg and 63 mg/kg were calculated for males and females, respectively. The lesions produced included degenerative changes and necrosis in the liver, spleen, exocrine and endocrine pancreas, kidney, salivary glands, myocardium skeletal muscle, bile ducts and other ducts. CPA produces focal necrosis in most organs at high doses and affects ducts or organs (such as the islets of Langerhans) originating from ducts at lower doses (Purchase, 1971).

18. In a study by Nishie, Cole and Dorner (1985) the effects of single doses of 0, 5, 7.5, 10, 11 and 12.5 mg/kg bw in groups of 5 – 26 mice on spontaneous motor activity were assessed. CPA caused a significant and dose-related reduction in the spontaneous motor activity (hypokinesia) at doses  $\geq 5$  mg/kg i.p. A slight reduction in motor activity was already noticeable 5 minutes after CPA injection, and maximum hypokinesia was reached in 30-60 minutes and returned to normal after about 2 hours or more depending upon the dose. This hypokinesia was associated with slow respiration and ptosis. This sedated condition resembled sleep, but the mice moved about intermittently with ptosis and retained a positive righting reflex. Cataleptic and hypothermic effects of CPA were monitored at 30 – 60 minute intervals in a group of 10 mice dosed with 10 mg/kg bw CPA. Peak cataleptic effects occurred after 60 minutes. Peak CPA-induced hypothermia occurred 30 minutes after injection and although body temperature increased it was still not back to normal after 7 hours. The mice tested for catalepsy and hypothermia, and survivors of the higher doses of CPA given in the LD<sub>50</sub> determination were kept for 1 week to monitor changes in body weight, gross neurological effects and death rate.

19. The LD<sub>50</sub> value was determined using groups of 9-12 mice given 11, 12.5, 13 or 14 mg CPA/kg. The lowest dose of CPA causing significant weight loss in mice was 7.5 mg/kg (1 day). The i.p. LD<sub>50</sub> of CPA was found to be  $13 \pm 0.05$  mg/kg. The

tremors induced by near-lethal doses of CPA were associated with voluntary or forced movements (action tremors), they worsened during the days following treatment, but they were weak compared with the exhausting and continuous tremors of the whole body caused by 20 mg tremorine/kg (used for comparison). When death occurred only 24-259 minutes after administration of CPA (11-14 mg/kg), it was preceded by dyspnoea cyanosis, opisthotonus and clonic leg movements and tonic extension of hind legs (convulsions). When death was delayed (2-6 days after CPA administration), it was preceded by prostration, ptosis, hypothermia, tremor and cessation of food and water intake resulting in cachexia, convulsions were not seen in this group of mice. CPA did not affect the rate of convulsion or death caused by either maximal electroshock or metrazol administration, but it did delay the onset of metrazol-induced seizures.

20. In rabbits 10 mg CPA/kg bw initially produced tachycardia, tachypnoea and sedation with an activated electroencephalogram. Of three rabbits given 10 mg CPA/kg, one died and, in this rabbit, slow delta waves were seen just before and during a brief period with clonic leg movements. In this animal death was accompanied by tonic extension of the hind legs, respiratory arrest and cardiac fibrillation (Nishie, Cole and Dorner, 1985).

21. Two acute toxicity studies were found in broiler chicks. Porter *et al.* (1988) saw significant ( $P \leq 0.05$ ) increases in brain dopamine and serotonin concentrations 96 hours after dosing chickens orally with CPA at 0.5, 5.0, and 10 mg/kg bw. The increases coincided with significant decreases in homovanillic acid and subtle increases of dihydroxyphenylacetic acid and 5-hydroxyindoleacetic acid concentrations. The brain weights of the treated birds were statistically insignificant from their respective controls, although increases in brain weight-body weight ratio within treatments and with time correlated with CPA toxicity. Venkatesh *et al.* (2005) randomly distributed broiler chicks into three groups of 12 birds. Groups were fed diets containing 10 mg/kg CPA, 1 mg/kg T2 toxin or no treatment. The CPA and T2 toxin treated groups showed significant ( $P < 0.01$ ) induction of apoptosis in the spleen and thymus, respectively, with peak induction at 24 h post-treatment. Additionally, a study on turkey poults demonstrated effects at 10 and 20 mg/kg/bw day, showing clinical symptoms, altered clinical pathology, and gross lesions (Miller *et al.* 2011).

22. The 96-hour median lethal dose of CPA injected i.p. into channel catfish *Ictalurus punctatus* (average weight, 19 g) was 2.82 mg/kg bw, with a 95% confidence interval of 2.48-3.12 mg/kg bw. The acute effects of CPA were characteristic of a neurotoxin. Some fish injected with CPA doses of 2.40 mg/kg bw or higher showed severe convulsions, tetany, and death within 30 minutes post injection. There were no lesions in the organs of the moribund fish examined grossly and histologically (Jantrarat and Lovell, 2011).

#### *Subacute/Repeat dose*

23. Wistar-derived rats were given weekly doses of 0, 12 or 21 mg CPA/kg bw in 1N-sodium bicarbonate, using an intragastric dose volume of 2.5 ml/kg bw, and subgroups of 8 (4 for controls) with equal numbers of males and females were killed 1 week after doses 2, 5, 9 and 14 had been administered. Males on the highest



dose level showed mild transient growth retardation in the first 4 weeks. Four rats died (with no gross pathological changes) suddenly during week 4. Three were from the higher dose group and one was from the lower dose group. No abnormal signs were observed in the surviving males or in any of the females throughout the 15 weeks of the experiment. Mild cellular degenerative changes were induced by CPA in the myocardium and in several other organs (kidneys, liver (but only in the high dose group at week 15), spleen, salivary glands, pancreas, male genital system and adrenal gland) where ballooning of nuclei, especially in ductal epithelia, was also characteristic. Generally, the changes noted were already evident after 2 doses of CPA and progressed slightly up to week 5. After this time the severity of some changes, was reduced. Some nuclear changes, however, especially in ductular epithelium such as that of the salivary glands, became more evident. The changes were only weakly related to dose level, sex and the number of doses given (van Rensburg, 1984).

24. Groups of 12 male SD rats received oral doses of CPA on 4 consecutive days at 0, 0.2, 2.0, 4.0, or 8.0 mg/kg/day. Only the 2 highest dose groups showed clinical signs of toxicity. Rats in the highest dose group exhibited abnormal behaviour, diarrhoea, rough coats, sunken eyes and other signs of toxicity after several days of dosing. Most of these rats were moribund before the last scheduled dose was administered. "Liver and spleen were more severely affected than other organs in the two highest dose groups. Livers contained diffuse pycnotic nuclei and, in some high-dose rats, focal areas of coagulative necrosis. In the high dose group aspartate and alanine aminotransferase activities were elevated, cytochrome P-450 concentration was decreased, and glutathione S-transferase activity was unchanged. Spleens were haemorrhagic and white pulp contained necrotic lymphocytes. White cell counts were decreased in a dose-related manner in the two highest dose groups. The GI tract of high-dose rats contained pycnotic nuclei, and sites of necrosis were observed in the stomach, but these lesions were limited to several animals, and were generally mild. Pathologic changes in conjunction with decreased feed and water intake probably contributed to the general deterioration of high-dose rats that resulted in death" (Morrissey, 1985).

25. A number of subacute/repeat dose studies in broiler chicks have been performed administering CPA by diet or by gavage. Typically, these have run 3 weeks from hatching with doses up to 50 mg/kg bw/day with one giving 100 mg/day per chick for 7 weeks. In each study observations are consistent, showing mortality at high doses; reduced bodyweight gain; immunosuppression; biomarkers of renal and hepatic injury as well as increased organ weights and pathological changes in multiple organs including the liver, spleen and kidneys. These adverse effects appear to show dose-dependency. Two studies show CPA to significantly interact with other mycotoxins (namely aflatoxin and T2) when administered (Gentles *et al.*, 1999; Cullen *et al.*, 1988; Dorner *et al.*, 1983; Kubena *et al.*, 1994; Smith *et al.*, 1992; Malekinejad *et al.*, 2011).

26. In a study by Morrissey *et al.* (1987) male rats were divided into 9 groups and were administered 0, 0.1 or 4.0 mg CPA/kg bw/day intragastrically (three groups per dose level) for three consecutive days. Thirty minutes after each of these CPA doses, aflatoxin B1 (AFB1) was administered to the rats by gavage at 0, 0.1 or 2.0 mg AFB1/kg bw/day. Of the 12 rats given each of these nine treatments, 6

were killed on day 4, after the initial dosing, and the rest were allowed a recovery period of 4 days prior to termination. All groups except those dosed with 2 mg/kg bw/day AFB1 gained weight. Weight loss in the three groups receiving 2.0 mg AFB1/kg/day occurred within 24 hours of the first doses. Feed consumption by these rats was about 60% of that in the other groups. The groups with a 4 week recovery period which had received 2 mg/kg bw/day AFB1 and high dose CPA had higher feed consumption (75 % controls) than those with low dose or no CPA (50 % controls). By the end of the recovery period, rats in these three groups had lost an average of 31-38 g of bodyweight.

27. Gross pathological findings were primarily limited to rats in the high AFB1 group. Prior to termination some of the rats receiving high CPA, in addition, were jaundiced. All groups with high AFB1 had shrunken liver and lesions in the kidney at the cortico-medullary junction. Microscopic changes were characteristic of aflatoxicosis in rats. At both dose levels CPA produced swollen endoplasmic reticulum (ER), and at the high dose, a loss of ribosomes from the ER. Glycocholic acid assays indicated liver damage only in those groups that received the high AFB1 dose. The authors concluded that neither toxin potentiates the action of the other at the dose levels used in this study (Morrissey *et al.*, 1987).

28. Pier *et al.* (1989) divided guinea pigs into 4 groups of 8 or 9 animals which were dosed orally with 2.2 mg/kg CPA or 0.045 mg/kg AFB1<sup>1</sup> singly or in combination in gelatin capsules. Doses were calculated according to mean group weights at the start of the experiment. On the 3<sup>rd</sup> day of toxin treatments a sensitising agent was injected into the guinea pigs and on the 10<sup>th</sup> day phytohaemagglutinin (PHA) was administered. On the 11<sup>th</sup> day cutaneous induration to PHA was measured. On the 20<sup>th</sup> day a sensitising agent was again injected and delayed cutaneous hypersensitivity calculated on day 21.

29. Clinical signs of intoxication (reduced emotive behaviour and mild dehydration) were first seen on day 3. There was a marked loss in body weight in the combined group by the 7<sup>th</sup> day and 2 of these animals died on the 8<sup>th</sup> day. By day 20, 6/9 guinea pigs in this group had died or been euthanised when considered moribund. Gross pathologic changes were generally confined to the liver and were predominantly in animals dosed with aflatoxins. Histopathologic changes were most notable in the combined group and consisted of cytoplasmic vacuolation of hepatocytes. This group also showed moderate thymic atrophy and 1 animal showed signs of acute tubular necrosis of the kidney. CPA treated animals had a lower degree of vacuole changes in centrilobular hepatocytes. Intracutaneous injections of PHA showed a significant reduction in proliferative response in aflatoxin, but not CPA-treated animals. An apparently reduced response was achieved in the combined group but did not achieve statistical significance. Tests for delayed cutaneous hypersensitivity showed a significant difference between the responses of aflatoxin and CPA. Animals treated with CPA showed a greater response, but this was not statistically verified. AFB1 significantly suppressed the lymphoblastogenic response to PHA while CPA alone had no detectable effect. In combination, CPA appeared to neutralise the effect of AFB1 and restored the count to normal levels.

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<sup>1</sup> AFB2, AFG1 and AFG2 were also present. The mixture contained 50 mg B1, 25 mg G1, 2.5 mg B2 and 2.5 mg G2. The activity equivalence of AFB1 in the mixture was 79.3 %.

There were significant reductions in haemolytic complement titers in serum from the groups of animals that had received aflatoxin (Pier *et al.*, 1989).

30. In a study by Hill *et al.* (1986) 24 SD rats were separated into 4 groups of 3 male and 3 female rats each and dosed i.p. with 0.1, 1 and 5 mg/kg bw CPA once daily. The control group was given 0.5 ml of 1 N sodium bicarbonate. Effects of CPA on cell-mediated immunity were assessed by a delayed-type hypersensitivity test. Humoral immunity was also assessed by measuring antibody production after i.p. injection of sheep red blood cells (RBCs).

31. There were no clinical signs directly related to CPA, however 1 rat died in the 5 mg/kg group of diarrhoea, dehydration and weakness on day 28. Mean weight gains of the treated rats were lower than those of the controls, however the decrease was significant ( $P < 0.05$ ) only in the 5 mg/kg group. All rats in the 5 mg/kg group had livers that appeared large with round edges when compared to the controls and lower dose groups. Mean liver, kidney and spleen weights did not differ significantly between control and treated groups. Microscopic lesions of the liver and kidneys were present in all treatment groups. Total protein, albumin, packed cell volume, and haemoglobin values were not significantly altered by CPA. The 1 and 5 mg/kg groups had higher mean neutrophil counts and lower mean eosinophil and lymphocyte counts than controls. On days 3, 5 and 7 after sheep RBCs had been injected the geometric mean titers (GMT) were lower in treated rats than controls. By day 14 the GMTs of treated rats had exceeded the control group. Results of the delayed-type hypersensitivity test were inconclusive (Hill *et al.*, 1986).

32. In a study by Hinton *et al.* (1985) male SD rats were assigned to groups of 0, 0.2, 2, 4 and 8 mg/kg bw/day CPA with 8 animals in each group. Rats were dosed orally each day for 4 consecutive days by gastric intubation, fasted overnight on the fourth day and anaesthetised with ether and decapitated on day 5. Rats dosed with 8 mg/kg bw/day died prior to the fixation of tissues and are therefore not considered further. Some rats receiving 4 mg/kg bw/day exhibited non-specific toxic signs such as rough coat and sunken eyes, within 24 hours of the first dose. No clinical signs of toxicity were observed in other dose groups or controls. At 4 mg/kg bw/day there was a relatively large amount of cytoplasmic vesiculation which was not as apparent at lower doses. Every cell examined from the livers dosed with 2 and 4 mg/kg bw/day had this vesiculated appearance. Only about 25 % of the cells in the 0.2 mg/kg group were affected. Of 2 hepatocytes from the 0.2 mg/kg group 1 was similar in appearance to control cells and the other to hepatocytes from higher dose groups. There was dilatation of the ER in the affected cell with the formation of vesicles, apparently from the ER. Nuclei, bile canaliculi, and associated membranes all appeared normal at this and higher doses. Higher doses had increased dilatation of the ER and more extensive distribution of vesicles throughout the cytoplasm. There was a dose-related increase in the width of the ER and degree of vesiculation. At the high dose level, more ribosomes had been shed into the cytoplasmic matrix than at lower doses. Mitochondria of rats dosed with 2 or 4 mg/kg were swollen, with increased swelling at the higher dose. Mitochondrial membranes were intact in all sections examined. Lysing cells were only observed at 4 mg/kg (Hinton *et al.*, 1985).



33. In a study by Lomax *et al.* (1984), 5 to 6-week-old crossbred pigs were given CPA at oral doses of 0, 0.01, 0.1, 1 and 10 mg/kg bw/day for 14 days. Clinical signs observed by day 7 in pigs given 10 mg/kg bw were weakness, inactivity, anorexia, rough hair coats, and reduced body weights. These pigs also developed diarrhoea during week 2. The pigs given 1.0 mg/kg bw had roughened hair coats and were moderately inactive during week 2. At necropsy, lesions were observed only in pigs given 10 and 1.0 mg/kg bw of CPA. Gross lesions in 7/8 pigs dosed with 10 mg/kg bw were serosal and mucosal hyperemia, and haemorrhage throughout the small and large intestine. The pigs also had yellow, fibrin and necrotic cellular material in the lumen of the small intestine and pale livers, 2 with raised red foci on the surface of the hepatic lobes and extending into the hepatic parenchyma. The only gross lesion observed in pigs given 1 mg/kg bw was gastric ulceration, observed in only 1 pig. Microscopic gastroenteric lesions increased in severity with increasing dose. Lesions in pigs given 10 mg/kg body weight were necrotising gastroenteritis, focal hepatocellular necrosis, hepatic peripheral lobular fatty change, and focal renal tubular nephrosis with focal suppurative tubulointerstitial nephritis. Pigs given 1.0 mg/kg body weight of CPA had necrotising gastritis and villous blunting in the jejunum and ileum (Lomax *et al.*, 1984).

#### *Subchronic*

34. A study by Jaskiewicz *et al.* (1988) investigated the toxicity of CPA alone and in combination with AFB1 through 3 experiments in 16 vervet monkeys of both sexes. 1) 1 female monkey was given 1 mg/kg bw/day of CPA intragastrically and the dose was doubled every third day to eventually achieve 60 mg/kg bw/day. 2) Two males and 3 female monkeys (including female from experiment 1) were fed 20 mg/kg bw/day CPA. After 60 and 120 days 2 males and 1 female, and 2 females and a male and a female control animal, respectively were terminated. 3) Six monkeys received AFB1 0.1 mg/kg per day, 2 received only this, 2 received 1 mg/kg per day CPA in addition and 2 received 20 mg/kg per day CPA in addition and 2 control animals received solvent only. Results of experiments 1 and 2 showed low toxicity of CPA in non-human primates with mild changes of epithelial cells of the biliary and pancreatic ducts and renal medullary tubules, and minute tubular atrophy. More pronounced pathological changes were in hepatocyte rough endoplasmic reticulum, small vessels and myocardium. Combined treatment with CPA and AFB1 indicated lack of a synergistic cumulative effect of both toxins. Animals treated with AFB1 only or AFB1 and low dose CPA developed more advanced liver lesions and died earlier than those which also received high doses of CPA (Jaskiewicz *et al.*, 1988).

35. In a study by Voss *et al.* (1990) groups of 12 male SD rats were given oral (gastric intubation) doses of 0, 0.2, 0.6, 2.0 or 4.0 mg CPA/kg bw/day for 13 consecutive weeks. No dose-related mortality or morbidity occurred. General appearance, behaviour, body weight gain and food consumption of all groups were similar. CPA had no definite adverse hematologic or serum chemistry effects, although serum creatinine concentrations of rats given 2.0 and 4.0 mg CPA/kg bw/day were increased after 7 and 13 weeks. Histopathologic effects were confined to the stomach. Acute inflammation of the lamina propria and submucosa of the glandular epithelium was found in 8/12, 11/12 and 11/12 rats dosed with 0.6, 2 and 4 mg/kg bw/day CPA, respectively. The neutrophilic infiltrate in

rats receiving 2 mg/kg bw/day or greater was mild to moderate intensity and minimal to mild intensity in animals given less than or equal to 0.6 mg/kg bw/day. No other dose-related microscopic lesions were found. Changes in hepatic ultrastructure were a subtle disruption of the cisternal pattern of the endoplasmic reticulum with ribosomal detachment in animals receiving 4 but not 2 mg/kg bw/day (Voss *et al.*, 1990).

36. Voss *et al.* (1990) repeated the 4 day study carried out by Morrissey *et al.* (1985) to compare the toxicity of the batches of CPA used. Rats were dosed with 8 mg/kg bw/day or vehicle, orally for 4 consecutive days. Clinical signs of toxicity and slight transient decreases in body weight were apparent in only 1 animal treated with 8 mg/kg bw/day CPA. The remaining 4 rats dosed with 8 mg/kg bw/day showed weight gain and food consumption comparable to controls. This suggested a difference in toxicity of the batches of CPA used. Voss *et al.* also noted that epimerisation of CPA occurs under basic conditions and differences in absorption and/or toxicity of CPA epimers may exist and influence the outcomes of studies (Voss *et al.*, 1990).

37. In a study by Nuehring *et al.* (1985), CPA in gelatin capsules was administered to five groups of dogs of unknown age and breeding status twice a day for 90 days at 0, 0.05, 0.25, 0.5, and 1.0 mg/kg bw/day (final doses are 0, 0.1, 0.5, 1 and 2 mg/kg bw/day). All dogs administered the 0.5 and 1.0 mg of CPA/kg doses and 1 dog given the 0.25 mg of CPA/kg dose died or were humanely killed before the scheduled termination of the study. Clinical signs of intoxication were not observed in the remainder of the dogs during the 90 days trial. Clinical signs of intoxication appeared 2 to 44 days after dosing was started and consisted of anorexia and, in 1 to 2 days, vomiting, diarrhoea, pyrexia, dehydration, weight loss, and CNS depression. Grossly, the entire alimentary tract had diffuse hyperemia with focal areas of hemorrhage and ulceration. Other lesions were renal infarcts, necrotising epididymitis, and ulcerative dermatitis. Microscopic lesions included ulceration, necrosis, vasculitis, lymphoid necrosis, karyomegaly in several organs including the liver, kidneys, bladder and skin, and decreased mitotic activity in intestinal crypt epithelium. Ulcerative and necrotic lesions were usually associated with vascular lesions. Clinical pathologic changes were increased numbers of white blood cells, neutrophils and monocytes and a decrease in the number of lymphocytes, and increased serum alkaline phosphatase activity (Nuehring *et al.*, 1985).

38. There are some uncertainties which may limit the usefulness of this study. Each treatment group had 5 dogs, with an approximately even split of female and male animals (actual numbers not given). Two dogs were pregnant during the study period, two females had been spayed and two dogs had reoccurrence of pneumonia which was present pre-treatment. Being reproductively intact appeared to have a protective effect for the female dogs and so the susceptibility of the females in each group varied. One dog in the 0.5 mg/kg bw/day group showed effects the authors attribute to CPA, however this is one of the two dogs (the other being in the 1 mg/kg day group) which suffered from a recurrence of pneumonia during the trial. The authors offer that this is dose-related as CPA may have an immunosuppressive effect. Owing to these factors this study may not be suitable to derive a lowest adverse effect level (LOAEL) based departure point. The lowest dose group

of 0.1 mg/kg bw/day had no pre-treatment issues and showed no dose related effects. Therefore, the no observable adverse effect level (NOAEL) of 0.1 mg/kg bw/day for this study may be suitable for the derivation of a health-based guidance value (HBGV).

39. CPA fed for 10 weeks at a concentration of 0.1 mg/kg of diet had a growth-suppressing effect ( $P < 0.05$ ) on channel catfish (average bodyweight, 7.5 g), and a concentration of 10 mg/kg caused accumulation of proteinaceous granules in renal tubular epithelium and necrosis of gastric glands. CPA had no effects on haematocrit or haemoglobin concentration, and erythrocyte and leukocyte counts ( $P > 0.05$ ) (Jantrarotai and Lovell, 2011).

40. There were two sub-chronic studies in broiler chicks. Kamalavenkatesh *et al.* (2004) found that CPA at 10 mg/kg and T2 at 1 mg/kg in feed either individually or in-combination adversely affected the health of chickens as evident from their body-weight gains. Diets containing up to 50 mg/kg CPA were fed to chickens for 28 days resulting in a grossly, yellow discolouration and granulomas of liver, diphtheritic ingluvitis and proventricular mucosal thickening. Microscopic changes included effects in the liver, blood vessels and spleen. These changes were dose related (Balachandran *et al.* 1998).

#### *Chronic*

41. No chronic studies for CPA were identified in the literature search.

#### *Reproductive/Developmental Toxicity*

42. Morrissey, Cole and Dorner (1984) randomly assigned sperm-positive female Fischer rats to 1 of 4 dose groups. Daily doses of 0, 1, 5 or 10 mg/kg bw CPA in 1N sodium bicarbonate were given by gastric intubation to 64 females on days 8 – 11 of pregnancy and to 53 females on days 12 – 15 of pregnancy. Sacrifice took place on day 21 of pregnancy. Dams in both groups receiving 10 mg/kg bw CPA showed signs of toxicity including rough fur coats and diarrhoea. One rat of each group died with additional signs including uncoordinated movements, inability to maintain posture, closed eyes and decreased feed consumption. In both groups there was no dose effect on mean body weight gained and no effect on the number of pregnant animals. There were no significant differences in pup weights, percentage pre- or post-implantation losses, or fetal deaths, compared to controls. Skeletal malformations and aberrations were present in pups from dams treated with 10 mg/kg bw but these defects and variations were not significantly increased compared to controls. Significant differences in skeletal development included retardation of ossification of cervical centra (d 12-15) and caudal vertebrae (d 8-11) in the two highest dose groups. Retardations of development were the most common manifestations of embryotoxicity. There were no statistically significant post-mortem gross pathologic findings in dams sacrificed at term. Although high dose rats had abnormal livers and spleens with coagulative necrosis and single cell necrosis. The authors concluded that since significant maternal toxicity occurred at the highest dose level in the absence of fetal malformations, the teratogenic potential of CPA is low (Morrissey, Cole and Dorner, 1984).

43. Nishie, Cole and Dorner (1987) determined an approximate oral LD<sub>50</sub> using 16 non-pregnant mice (4 per dose) which were maintained for 7 days. These animals were also checked for body temperature, spontaneous motor activity and pain reflex time. The approximate oral LD<sub>50</sub> of nonpregnant mice was 63 ± 4.4 mg/kg bw. The clinical signs preceding death are identical to those observed with i.p. administration (Nishie *et al.*, 1985): hypothermia, ptosis, gait disturbance, hypokinesia, dyspnoea and action tremor. Delayed death was preceded by prostration and cessation of water and feed intake. In nonpregnant mice surviving near lethal doses (50, 60, 70 mg/kg bw, orally) the oestrous cycle returned at the expected time interval (Nishie, Cole and Dorner, 1987).

44. Pregnant mice were dosed (15, 20, 30, 45 or 50 mg/kg) with CPA in the early phase of pregnancy (day 2-8). Male mice used in this study were untreated. A limited number of pregnant mice were treated with 66 mg/kg ergonovine maleate (orally, subcutaneous) to compare its effect with that of an equivalent dose of CPA (50 mg/kg). Among control sperm-positive mice treated with oral 1 M sodium bicarbonate solution, 97.5% were gravid on necropsy day (pregnancy day 12). A single oral dose of CPA (15-50 mg/kg) given on days 2 to 8, decreased the pregnancy rates significantly. In general, the pregnancy rates decreased with increasing dose of CPA. In groups treated with a single dose of 50 mg/kg CPA on pregnancy day 4 to 8, vaginal haemorrhage was observed 1 to 7 days after treatment, and it usually resulted in termination of pregnancy (abortion). Fetal resorption rates were higher than the control rate only in the groups treated with 30 mg/kg CPA on day 4 or 8. CPA decreased body weight gains and the weights of uteri with fetuses. The ovary weights were generally not changed. Ergonovine maleate (66 mg/kg) had no significant effect on all of the parameters examined (Nishie, Cole and Dorner, 1987).

45. Khera *et al.* (1985) randomly assigned 15 to 20 mated females per test group (4, 8 or 16 mg/kg bw in 1 N sodium bicarbonate) and a control group. Doses were administered once daily by oesophageal intubation for 4 consecutive days from days 9-12 of pregnancy. Necropsy occurred on day 19 of pregnancy. There were no overt signs of toxicity or body weight suppression in dams during pregnancy or at necropsy at daily doses of up to 16 mg/kg bw CPA. A slightly decreased incidence of pregnancy was observed at all doses but was not dose-related or statistically significant. At all test doses, the incidence of live, runted and dead fetuses, resorptions and male/female fetuses were within the control range. Mean fetal weight failed to show a dose-related effect. The number of malformations and aberrations in fetuses of all treated groups were not statistically different from control values (Khera *et al.*, 1985).

### Genotoxicity

46. Sorensen (1984) tested 0.01, 0.03, 0.1, 0.3 and 1.0 micro-mole per plate (µmol/plate) CPA, with and without S9 activation in *Salmonella typhimurium* strains TA98 and TA100. CPA was mutagenic to *S. typhimurium* TA98 and TA100 strains in the presence of metabolic activation. The correlation coefficients between concentration and the number of revertants per plate were 0.974 and 0.898 with metabolic activation for TA98 and TA100, respectively. In the absence of metabolic activation there was no correlation between concentration and mutagenic response.

A specific activity of 140.74 revertants/ $\mu$ mol was estimated for CPA, compared to a specific activity of 6.24 revertants per pico moles (revertants/pmol) or 6,240 revertants/ $\mu$ mol for AFB1 (Sorensen, Tucker and Simpson, 1984).

47. In a study by Kuilman-Wahls (2002) CPA was applied at 225, 450 and 900 nmol/plate in the Ames test, using TA98 and TA100 *Salmonella typhimurium* strains. CPA was not mutagenic in the presence or absence of metabolic activation by S9-liver fractions obtained from Arochlor treated rats. However in the presence of AFB1, CPA was shown to dose-dependently inhibit the mutagenic activity of AFB1 (Kuilman-Wahls *et al.*, 2002).

48. *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 were incubated with 0.25, 2.5, 25 and 250  $\mu$ g/plate in the presence and absence of S9-mix obtained from Arochlor treated rats. There was no mutagenic activity for any of the CPA concentrations in the presence or absence of S9 (Wehner *et al.*, 1978).

## HBGV

49. There is currently no HBGV for CPA.

## Exposure Assessment

50. Following a request from members, a literature search was performed to identify papers sampling for CPA in infant formula and breast milk. No relevant papers were identified.

51. CPA was measured in the 2014 TDS – mycotoxins analysis (FSA, to be published). Most food samples analysed were below the limit of detection (LOD) (0.5  $\mu$ g/kg) or between the LOD and the limit of quantification (LOQ). “Brown bread” and “herbs and spices” samples contained levels of 0.79 and 0.89  $\mu$ g/kg, respectively which are below the LOQ but above the LOD. One sample, “other snacks, not potato” contained CPA at 4.27  $\mu$ g/kg. There are currently no limits for CPA in foods in legislation. Exposures were calculated using data from the total dietary survey (TDS) and consumption data from the diet and nutrition survey of infants and young children (DNSIYC) and the national diet and nutrition survey (NDNS) (Table 1).

52. Mean and 97.5th percentile exposures for infants aged 4 to 12 months ranged from 0 – 0.004 and 0.001 – 0.011  $\mu$ g/kg bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile exposures ranged from 0.001-0.007 and 0.005 – 0.018  $\mu$ g/kg bw/day. Calculated mean and 97.5th percentile dietary exposures for young children aged 18 to 60 months ranged from 0.002-0.009 and 0.007 – 0.022  $\mu$ g/kg bw/day.



**Table 1. Estimated CPA chronic dietary exposures from the TDS for humans aged 4 to 60 months**

<b>Age Group</b>	<b>Mean (µg/kg bw/day)*</b>	<b>97.5<sup>th</sup> percentile (µg/kg bw/day)*</b>
4 to <6 month-olds (n=116)	0.000-0.001	0.001-0.003
6 to <9 month-olds (n=606)	0.000-0.002	0.003-0.009
9 to <12 month-olds (n=686)	0.001-0.004	0.004-0.011
12 to <15 month-olds (n=670)	0.001-0.006	0.005-0.015
15 to 18 month-olds (n=605)	0.001-0.007	0.007-0.018
18 to 24 month-olds (n=118)	0.002-0.009	0.008-0.019
24 to 60 month-olds (n=688)	0.002-0.009	0.007-0.022

\* Exposures given as a range of lower bound to upper bound estimates

## Risk characterisation

53. Lomax *et al.* (1984) was considered for derivation of a HBGV, however it was felt that as extrapolation from sub-acute to chronic exposure would be required, Nuehring *et al.* (1985) would be more appropriate. Nuehring *et al.* (1985) has a number of limitations, however it demonstrates a clear reduction in dose-response. The lowest dose group, 0.1 mg/kg bw/day shows no dose related effects and is not hampered by any of the overt pre-treatment issues affecting the other groups.

54. No dose related effect was seen in dogs dosed with 0.1 mg/kg bw/day in the sub-chronic dietary study by Nuehring *et al.* (1985). This NOAEL has been selected for a margin of exposure (MOE) risk assessment approach. Table 2 shows the margins of exposure calculated using the upper bound estimate of exposure as given in Table 1 for each age group and for both the mean and 97.5<sup>th</sup> percentile.

Table 2. Margin of exposure values for humans aged 4 to 60 months based on upper bound exposures

<b>Margin of Exposure (MOE)*</b>		
<b>NOAEL 0.1 mg/kg</b>	<b>Mean intake</b>	<b>High intake (97.5<sup>th</sup> percentile)</b>
4 to <6 month-olds (n=116)	100,000	33,300
6 to <9 month-olds (n=606)	50,000	11,100
9 to <12 month-olds (n=686)	25,000	9,100
12 to <15 month-olds (n=670)	16,700	6700
15 to 18 month-olds (n=605)	14,300	5600
18 to 24 month-olds (n=118)	11,100	5300
24 to 60 month-olds (n=688)	11,100	4500

\*MOE given to nearest 100

## Conclusions

55. The MOE's generated using upper bound exposures range from 4,500 to 100,000. The margin between the NOAEL of 0.1 mg/kg bw/day from the Nuehring study and estimated UK exposures are big enough to suggest that CPA present in the diet does not pose a health concern for infants aged 0 to 12 months and children aged 1 to 5 years.

## Questions on which the views of the Committee are sought

56. Members are invited to consider the following questions:

- i) Are Members happy with the use of the Nuehring study NOAEL as the basis for the MOE calculations?
- ii) Do Members have any other comments on this paper?
- iii) Do Members feel this work should be presented as a statement, or should it be included in the mycotoxins addendum?

**Secretariat**

**October 2019**

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