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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. 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).

2. After ingesting CPA-contaminated feeds, test animals display gastrointestinal 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 ADME and toxicity of CPA are provided in the relevant sections below.

3. 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

4. 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).

5. 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.

6. 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 mg/kg bw/day over 14 consecutive days. The authors reported a NOEL of between 0.01 and 0.1 mg/kg bw/day because lesions were observed in the gastrointestinal tract at dose levels as low as 0.1 mg/kg bw/day.

7. 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 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.

8. 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 TDI.

9. De Waal (2002) proposed a TDI of 0.1 µg/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. Dose-dependent signs of vascular toxicity (mainly in the gastrointestinal tract, liver and kidneys) was the critical effect observed at the 0.5 and 2.0 mg/kg bw/day dose levels.

10. 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

11. In a study by Byrem et al., (1999) a single bolus injection of 20 mg CPA (in 2 ml 1 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 ± 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 kg ± 0.23 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 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 ± 44 ng/mL and in skeletal muscle were 469 ± 86 ng/g. (Byrem et al., 1999).

12. [^{14}C] CPA was administered to Sprague-Dawley rats intragastrically (5 mg/kg, 0.6 µCi/kg) and parenterally (1 mg/kg, or 0.12 µCi/kg) (Norred, 1990) or orally (5 mg/kg, 0.6 µCi/kg) (Norred et al., 1985). The biological half-life of [^{14}C] was 33 – 43 hours, depending on route of administration. Radioactivity was not excreted into expired 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. 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

13. In a study by Purchase (1971) ip 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. An LD_{50} 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_{50}'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)

14. 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 group 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 ≥ 5 mg/kg ip. 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_{50} determination were kept for 1 week to monitor changes in body weight, gross neurological effects and death rate.

15. The LD_{50} 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 ip LD_{50} of CPA was found to be 13 ± 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 min after administration of CPA (11-14 mg/kg), it was preceded by dypsnoea 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.

16. 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, 1 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).

17. In a study by Porter et al. (1988) chickens dosed orally with CPA at 0.5, 5.0, and 10 mg/kg bw showed significant ($P \leq 0.05$) increases in brain dopamine and serotonin concentrations 96 hours after dosing. The increases coincide with significant decreases in homovanillic acid (HVA) and subtle increases of dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5HIAA) 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. No significant changes were observed in dopamine, DOPAC, HVA, serotonin, and 5HIAA concentrations among the treatments at 3, 24, and/or 48 hours after dosing. (Porter et al.,1988).

18. Venkatesh et al. (2005) randomly distributed 28-day old broiler chicks into three groups of 12 birds. Groups were fed diets containing no treatment (control), 10 mg/kg CPA or 1 mg/kg T2 toxin to determine the mechanism of cell death in spleen and thymus at 6, 12, 24, and 36 hours post-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. These findings were confirmed ultrastructurally and with semi-thin sections of the spleen and thymus stained with toluidine blue.

19. The 96-hour median lethal dose of CPA injected ip 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. (Jantrarotai and Lovell, 2011).

20. Turkey poults acutely exposed to CPA at 5, 10, and 20 mg/kg bw/day exhibited clinical symptoms, altered clinical pathology, and gross lesions. Clinical signs included lethargy, ataxia, drooped head and wings, ruffled feathers, regurgitation, marked anorexia, decreased amount of and unformed faeces. Birds given 10 or 20 mg/kg bw/day had decreased total serum protein and albumin concentrations. Poults given 10 or 20 mg/kg bw/day had proventriculus and ventriculus focal erosions, a fibrinogelatinous membrane present in the ventriculus, and increased liver weights. Turkeys given 5 mg/kg bw/day appeared healthy, with no change in body weight gain, no gross lesions or altered clinical chemistry. (Miller et al. 2011)

Subacute/Repeat dose

21. Wistar-derived rats were given weekly doses of 0, 12 or 21 mg CPA/kg bw in 1 N-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 1 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)

22. 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 gastrointestinal 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).

23. In a study by Gentles et al. (1999) the individual and combined effects of OTA and CPA were evaluated in Petersen × Hubbard broiler chickens from 1 day to 3 weeks of age. The experimental design was a 2 × 2 factorial with treatments of 0 and 2.5 mg OA/kg feed and 0 and 34 mg CPA/kg feed. CPA had a significant effect on body weight gain by the end of week 1 (11 % decrease compare to controls). For OTA and combination there was no significant change. Body weight gain was reduced ($P < 0.05$) by OA, CPA, and OA-CPA in combination at the end of 3 weeks. OTA significantly increased the relative weight of the kidney and serum uric acid and triglycerides and declines in total protein, albumin, and cholesterol were observed. Increased relative weights of the pro-ventriculus and increased activity of creatine kinase were the effects of CPA. OA-CPA toxicity was characterised by increased relative weights of the liver, kidney, pancreas, and proventriculus; decreased concentrations of serum albumin, total protein, and cholesterol; increased activity of creatine kinase; and increased concentrations of triglycerides and uric acid. Postmortem examination revealed that the chickens fed CPA or OA-CPA had thickened mucosa and dilated proventricular lumen. (Gentles et al., 1999)

24. Cullen et al. (1988) dissolved CPA in corn oil which was administered by gavage to broiler chicks daily, from the day of hatching for 23 days. Chicks were assigned to 4 groups (0, 1, 2, or 4 mg CPA/kg bw in corn oil). Each group was composed of 10 male and 10 female chicks. Surviving chicks were euthanatised and necropsied on day 24. In the group dosed with 4 mg/kg bw CPA 5/20 chicks died before day 23. Histologic examination revealed that the most common lesions consisted of necrosis and hemorrhage or hyperplasia of the mucosa of the proventriculus (up to 3 times normal depth) and hepatocellular vacuolation. Skeletal muscle degeneration, characterised by myofiber swelling or fragmentation accompanied by an infiltrate of macrophages and heterophils, was detected in the group given 4 mg/kg bw. This degeneration was associated with an increase of plasma creatine kinase activity. Focal hepatocellular and splenic necrosis also developed in the groups given 4 mg/kg bw. (Cullen et al., 1988).

25. Dorner et al. (1983) artificially contaminated chicken rations with purified CPA at concentrations of 10, 50, and 100 µg/g and fed ad libitum to eight groups of chickens for 7 weeks. Chickens receiving feed with 100 µg/g of CPA had high mortality, decreased weight gain, and poor feed conversion when compared with birds receiving other doses. Post-mortem examination showed that chickens fed the two greatest doses of CPA had proventricular lesions characterised by mucosal erosion and hyperemia (100 µg/g) and by thick mucosa and dilated proventricular lumens (50 µg/g). Birds given 100 µg/g of CPA in feed also had numerous yellow foci in their livers and spleens. Microscopic examination of tissues of birds that received 100 µg/g of CPA revealed ulcerative proventriculitis, mucosal necrosis in the gizzard, and hepatic and splenic necrosis and inflammation. Birds given 50 µg/g of CPA had microscopic lesions in the proventriculus (characterised by chronic mucosal inflammation and hyperplasia of the proventricular mucosal epithelium), liver, spleen and heart, however these lesions were generally more focal and less

tissue destructive. Birds given 10 µg/g of CPA and control birds had no significant treatment-related lesions. (Dorner et al., 1983).

26. In a study by Kamalavenkatesh et al. (2005) forty, newly hatched, unsexed broiler chicks were fed diets containing 10 mg/kg CPA and 1 mg/kg T2 toxin (T2) either individually or in combination for 28 days to study the immunopathological effects. Thymic atrophy and petechiae were observed in all toxin-fed birds. Necrotic foci were observed in the spleen of CPA fed birds. Lymphoid organs revealed lymphocytolysis and lymphoid depletion in all toxin fed birds. Changes in lymphocyte subsets in the thymus and spleen were assessed by measuring numbers of $CD4^+$ and $CD8^+$ lymphocytes. Thymic and splenic $CD4^+$ and $CD8^+$ lymphocytes decreased significantly ($p<0.01$) in toxin fed birds when compared to the control. Thymic $CD8^+$ lymphocytes of T2 and CPA-T2 showed significant ($p<0.01$) decrease from that of CPA and control groups. Splenic $CD4^+$ and $CD8^+$ lymphocytes showed significant ($p<0.01$) decrease in CPA and CPA-T2 fed groups when compared to the control. The T2 group did not differ significantly from that of control. The stimulation index of splenocytes to concavalin A revealed a significant ($p<0.01$) decrease in all toxin fed birds. A significant ($p<0.01$) decrease was observed for the haemagglutination inhibition titres to Newcastle disease virus vaccine F strain of birds fed CPA, T2 and in combination. Significant ($p<0.01$) interaction was found for lymphocyte subsets, SI and HI titres to NDV. The study indicated the immunosuppressive effect of these toxins either alone or in combination in broiler chicks. (Kamalavenkatesh et al., 2005).

27. Kubena et al. (1994) investigated the effects of feeding 6 mg T2 toxin and 34 mg CPA/kg of diet singly and in combination were characterised in male broiler chicks from 1 day to 3 weeks of age. Body weights were depressed by CPA (from week 1 to 3) and T2 and the combination of T-2 and CPA (weeks 2 and 3). When compared with controls, relative weights of the kidney and pancreas were significantly increased only in the T2-CPA group, the relative weights of the proventriculus increased for CPA alone or in combination with T2, relative weights of the gizzard were increased in T2 alone or in combination with CPA, relative weights of the bursa were decreased in T2 alone, CPA alone or T2-CPA combination and the relative weights of the liver, heart and spleen were not altered by any of the treatments. There was a significant synergistic interaction between T2 and CPA for relative liver and kidney weights and serum cholesterol and triglyceride concentrations and a significant interaction between T2 and CPA for 3-week body weights and relative bursa of Fabricius weights, which were less than additive.

28. Dietary treatment effects on serum biochemical values, serum enzyme activities and haematology values varied. Neither the efficiency of feed utilisation nor mortality was affected by dietary treatments. Oral lesions were present in a majority of the chicks fed diets containing T2 with or without CPA. When compared with controls, other variables measured exhibited additive or less than additive toxicity. These data demonstrate that T-2 and CPA alone and in combination can cause reduced performance and adversely affect broiler health. The effects of these mycotoxins may be exacerbated by other factors when under field conditions; hence, the potential detrimental effects of these two mycotoxins when present alone or in combination cannot be dismissed. (Kubena et al., 1994).

29. 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.

30. 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)

31. 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[1] singly or in combination in gelatin capsules. Doses were calculated according to mean group weights at the start of the experiment. On the 3rd day of toxin treatments a sensitising agent was injected into the guinea pigs and on the 10th day phytohaemagglutinin (PHA) was administered. On the 11th day cutaneous induration to PHA was measured. On the 20th day a sensitising agent was again injected and delayed cutaneous hypersensitivity calculated on day 21.

32. 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 7th day and two of these animals died on the 8th 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

[1] 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 %.

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

33. Smith et al. (1992) arranged eighty 1 day old male broiler chicks into 4 dosing groups of control, 3.5 mg/kg diet AF, 50 mg/kg diet CPA and 3.5 mg/kg AF and 50 mg/kg CPA for 3 weeks. The treatments were replicated 4 times. All treatments significantly reduced bodyweights. The reduction in bodyweight of birds dosed with AF alone in the 3rd week was 12.5 %, in birds dosed with CPA at weeks 1, 2 and 3 was 22.5, 20.5 and 32.6 %, respectively and those on combined diet at weeks 1, 2 and 3 was 15.8, 33.2 and 35.6 %, respectively. One bird died in each of the AF and CPA treatment groups and 3 birds died in the combined group. The relative liver weights were significantly increased by CPA alone and the combination. The relative weight of the kidney was significantly increased by all treatment groups. The relative pancreas weight was significantly increased only in the combination group. CPA alone and the combination significantly increased the relative weight of the proventriculus. The relative weight of the gizzard was not affected by any group and the relative bursa of Fabricus weights were significantly reduced by CPA alone and the combination. Values for serum total protein, albumin and cholesterol were significantly lower in the AF group than in controls, but values for uric acid and cholesterol in the CPA group were higher than controls. Significant interaction of AF and CPA altered serum albumin concentration. Serum glutamic transaminase activity was significantly increased by CPA alone, serum triglyceride values for AF were significantly lower than controls and other groups. Lactate dehydrogenase was significantly decreased by AF. AF and CPA singly and in combination lowered levels of serum phosphorous significantly when compared to controls. Blood urea nitrogen was significantly increased by AF and the combination over controls. (Smith et al., 1992).

34. 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 ip with 0.1, 1 and 5 mg/kg bw CPA once daily. The control group was given 0.5 ml of 1N 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 ip injection of sheep red blood cells (RBCs).

35. 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)

36. Malekinejad et al. (2011) divided 10 day old male broiler chicks into control and 10, 25 and 50 µg/kg CPA groups. CPA doses or control (saline) was given daily by crop gavage for 28 days. Body weight gain, serum level of alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), uric acid, creatinine, and blood urea nitrogen (BUN) were measured after 2 and 4 weeks exposure. Moreover, the total thiol molecules (TTM) and malondialdehyde (MDA) content of the liver and kidneys were assessed.

37. No birds died in any of the control or treatment groups. There were no significant differences in body weight gain between the control and test groups after 28 days. The authors were unable to collect the whole kidney from birds and weigh them, therefore only liver weights were assessed. After 28 days, elevation of hepatic weight was significant in the 25 and 50 µg/kg groups in what appeared to be a dose-dependent response. ALP and GGT were significantly increased in 25 and 50 µg/kg groups at 28 days compared to the controls. Serum uric acid, creatinine and BUN increased significantly in a time and dose-dependent manner. TTM content in both the kidney and liver was significantly reduced after 28 days exposure to 25 and 50 µg/kg CPA. After 28 days MDA content in the liver of birds dosed with 25 and 50 µg/kg was significantly higher than controls.

38. CPA-exposed birds showed dose-dependent pathologic alterations in the liver such as congestion, cell swelling, fatty degeneration, inflammatory cell infiltration, disintegration of the cells and necrosis. The necrotic reactions tended to appear around the bile ducts and were more apparent at the 50 µg/kg dose. The main pathological findings of the kidneys included tubular degeneration and necrosis, haemorrhages in the renal parenchyma, tubular cell swelling and ureter epithelial cells hyperplasia. (Malekinejad et al., 2011).

39. 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 cannaliculi, 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).

40. 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 hemorrhage 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

41. 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) One 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 animals, 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).

42. In a study by Voss et al. (1990) groups of 12 male Sprague-Dawley 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).

43. 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).

44. 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).

45. CPA fed for 10 weeks at a concentration of 100 µg/kg of diet had a growth-suppressing effect ($P < 0.05$) on channel catfish (average bodyweight, 7.5 g), and a concentration of 10,000 µg/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)

46. The effect of feeding diets containing CPA and T2-toxin at levels of 10 mg/kg and 1 mg/kg (respectively) on the growth rate in broiler chicken was assessed from 0 to 28 days of age. Body weights were recorded at weekly intervals. Highly significant ($p < 0.01$) differences were observed between the control and toxin treated groups in body weights. The CPA group differed significantly ($p < 0.01$) from other treatment groups and control. No significant difference was observed between the T2 and CPA-T2 groups. There was a significant ($p < 0.01$) reduction in body-weight gain in toxin-treated groups when compared to the control. But, no significant difference was observed between the T2-group and CPA-T2 group in body-weight gain. Highly significant ($p < 0.01$) CPA-T2 interaction was also observed. It was concluded that CPA and T2 either individually or in-combination adversely affect the health of broiler chicken as evident from their body-weight gains. (Kamalavenkatesh et al. 2004)

47. Diets containing 0, 12.5, 25 and 50 mg/kg CPA were fed to 348 Vencob broiler chickens for 28 days. Nine birds from each treatment were sacrificed at weekly interval for pathological study. Grossly, yellow discolouration and granulomas of liver, diphtheritic ingluvitis and proventricular mucosal thickening were observed in CPA fed birds. Microscopically, liver showed degeneration, necrosis, fibrosis, microgranulomas, acinar formation and dysplasia. Proventricular mucosal hyperplasia and necrosis, cornification of crop mucosa, diphtheritic ingluvitis and ventriculitis, enteritis, splenic lymphoid depletion and glandular transformation of bursal follicles were observed. These changes were dose related. (Balachandran et al. 1998)

Chronic

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

Reproductive/Developmental Toxicity

49. 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 1 N 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 clinical signs of toxicity of 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 postimplantation 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 singe 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).

50. Nishie, Cole and Dorner (1987) determined an approximate oral LD_{50} using 16 nonpregnant 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_{50} of nonpregnant mice was 63 ± 4.4 mg/kg bw. The clinical signs preceding death are identical to those observed with ip administration (Nishie et al., 1985): hypothermia, ptosis, gait disturbance, hypokinesis, dyspnea 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).

51. 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 hemorrhage 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).

52. 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).

53. The presence of CPA as a mycotoxin has been reported in feed and foodstuffs. The aim of this investigation was to determine the effects of CPA on reproductive functions of male mice. In this experiment, 40 mature male mice were randomly assigned into five groups (n=8): control, control-sham, CPA (0.03 mg/kg bw), CPA (0.06 mg/kg bw) and CPA (0.12 mg/kg bw). Following 28 days exposure to CPA, sperm quality parameters, in vitro fertilisation (IVF) capacity of sperms, serum testosterone level, Leydig cells number and serum total antioxidant capacity (TAC)

were analysed. The results revealed a significant ($P<0.05$) reduction in sperm count, sperm viability, sperm motility, chromatin quality of sperm, sperms with intact DNA, IVF rate, testosterone level, Leydig cell distribution and TAC in comparison to the control group. The most prominent detrimental effects of CPA were found at the highest given dose level. (Bonyadi et al., 2018).

Genotoxicity

54. Sorensen (1984) tested 0.01, 0.03, 0.1, 0.3 and 1.0 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/µmol was estimated for CPA, compared to a specific activity of 6.24 revertants/µmol for AFB1. (Sorensen, Tucker and Simpson, 1984).

55. 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).

56. S. typhimurium strains TA98, TA100, TA1535 and TA1537 were incubated with 0.25, 2.5, 25 and 250 µg/plate in the presence and absence of S9-mix obtained from Aroclor 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

57. There is currently no HBGV for CPA.

Exposure Assessment

58. Most food samples analysed were below the LOD (0.5 µg/kg) or between the LOD and LOQ . “Brown bread” and “herbs and spices” samples contained levels of 0.79 and 0.89 µg/kg, respectively which are below the LOQ but above the LOD. One sample, “other snacks, not potato” contained CPA at 4.27 µg/kg. There are currently no limits for CPA in foods in legislation. Exposures were calculated using data from the TDS and consumption data from DNSIYC and NDNS (Table 1).

59. Mean and 97.5th percentile exposures for infants aged 4 to 12 months ranged from 0 – 0.004 and 0.001 – 0.011 µg/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 µg/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 µg/kg bw/day.

Table 1. Estimated CPA chronic dietary exposures from the TDS for humans aged 4 to 60 months (µg/kg bw/day) (LB-UB)

Age Group	**Mean**	**97.5th percentile**
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

Risk characterisation

60. There is currently no HBGV against which to compare the estimated exposures presented in Table 1. Most food samples analysed were below the LOD (0.5 µg/kg) or between the LOD and LOQ.

Conclusions

Questions on which the views of the Committee are sought

61. Members are invited to consider the following questions

i). Do Members think that data from these studies could be used to derive an HBGV? And if so are there any studies for which Members would like more details provided?

ii). A number of the published studies are in broiler chickens. How relevant is this for humans?

iii). Is there any other information that Members would like provided?

Secretariat

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