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

Review of potential risks from 4, 15-diacetoxyscirpenol in the diet of infants aged 0 to 12 months and children aged 1 to 5 years

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. A scoping paper (TOX/2015/32)[1] "COT contribution to SACN review of complementary and young child feeding; proposed scope of work for 1-5 year old children" was reviewed by the COT in 2015. A further scoping paper for mycotoxins was presented to the COT in 2017[2]. This discussion paper is a review of 4, 15-DAS as the EFSA Opinion has recently been published and can be reviewed along with the JECFA evaluation.

3. In a request from the European Commission, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) was asked to assess whether 4,15-diacetoxyscirpenol (DAS) in food and feed is a potential risk for public and animal health, considering the toxicity of DAS and the occurrence in food and feed. Possible interactions with other *Fusarium* toxins, in particular group A trichothecenes, as regards toxicity and occurrence should be included in the evaluation.

4. This discussion paper has considered exposures based on concentration data measured in the mycotoxins Total Diet Study (TDS) by the Food and Environment Research Agency (FERA).

5. 4,15-DAS is a type A trichothecene mycotoxin from the *Fusarium* species group. It is found in cereals and cereal-based products including

[1] COT scoping paper (TOX/2015/32) available at: https://cot.food.gov.uk/sites/default/files/TOX2015-32%20Feeding%20Review%20Scoping%20Paper.pdf

[2]COT mycotoxins scoping paper available at: https://cot.food.gov.uk/sites/default/files/tox2017-30_0.pdf

wheat, barley, rice, rye, maize, oats and sorghum. In addition, it has been found in coffee beans.

6. The highest levels have been reported for wheat, sorghum and coffee. DAS has been found to co-occur with many other mycotoxins in grains and grain-based products, in particular *Fusarium* toxins including type A and B trichothecenes, and zearalenone.

7. 4,15 DAS had been assessed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2016 and, as requested by the European Commission, the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM) (2018).

8. The tolerable daily intake (TDI) of 0.65 µg/kg bw established by EFSA for 4,15-DAS[3], based on epidemiological data, was >10-fold higher than that established by JECFA. JECFA included 4,15-DAS in the group TDI for T2 an HT2 based on structural similarity. EFSA also calculated an acute reference dose (ARfD) of 3.2 µg/kg bw for 4,15-DAS.

9. This discussion paper provides a summary of the toxicokinetics and toxicity of 4,15 DAS, where data are available. Toxicity studies published since the 2016 JEFCA report and in the EFSA 2018 Opinion are also summarised or described. More detailed summaries of these toxicity studies are available in Annex A. The derivation of the health based guidance values (HBGVs) for each of the above evaluations is detailed. Exposure assessments have been carried out and risk characterisations and conclusions/discussion provided.

10. The CONTAM Panel considered that there is insufficient evidence to conclude on the transfer of DAS from feed.

Toxicokinetics

11. EFSA has reviewed the available data on Absorption, Distribution, Metabolism and Excretion (ADME) of 4,15 DAS.

12. *In vitro*, DAS is metabolised to a large number of metabolites. The main metabolic processes are deacylations, hydroxylations, deepoxidations and glucuronide conjugations. Deepoxidation reactions have primarily been found after incubation with GI content or faeces. After oral administration in rats and mice, the absorption of DAS has not been quantified but the excretion ratio in urine and faeces indicated high absorption. After absorption, DAS was rapidly distributed to most organs. Tissue concentrations decreased rapidly with no apparent accumulation in any tissue and more than 90% of radiolabelled DAS was excreted within 24 h, with an approximate 80% via urinary excretion. Only 2–3% of orally administered DAS was estimated to

[3] EFSA Scientific Opinion available at: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2018.5367

remain in the body after a few days. DAS was rapidly metabolised to a large number of metabolites *in vivo*. (EFSA, 2018).
13. No information is available of the transfer of DAS to breast milk.

Toxicity

14. The CONTAM Panel noted that the human risk of DAS in food had not been assessed by EFSA previously and therefore reviewed all available data from studies conducted in experimental animals and humans.

15. Only one scientific risk assessment on DAS in food and/or feed performed by national agencies or national and international independent expert advisory committees was identified by the CONTAM Panel; the recent assessment of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in November 2016 (FAO/WHO technical report 1002, 2017).

16. JECFA evaluated previously published toxicological data on humans and animals (the same as in the 2018 EFSA review) on DAS and concluded that neither a dose-effect relationship nor a point of departure could be derived from the limited available toxicological data.

17. The CONTAM Panel identified the same several studies which characterise the oral acute toxicity of DAS in rodents as well as acute toxicity after i.v. exposure in other experimental animal species which were tested in preclinical studies for the development of DAS (named anguidine) as a cytostatic anticancer drug. The oral, intraperitoneal (i.p.) and the i.v. routes were considered relevant to describe the acute toxicity.

Summary of animal toxicity studies

18. DAS showed acute toxicity in rodents with oral LD_{50} ranging from 2.1 to 15.5 mg/kg bw. Haematological effects such as anaemia, leukopenia and thrombocytopenia have been observed. When administered i.v., the LD_{50} resulted to be slightly lower, ranging from 1 to 12 mg/kg bw. The oral repeated dose toxicity studies performed in rodents are very few and not sufficiently described to be used for identifying a reference point (RP) for the hazard characterisation. However, in guinea pigs, organs with a high rate of cell proliferation such as oesophagus mucosa, small intestine, haematopoietic bone marrow have been identified as a target of adverse effects of DAS; emesis[4] was also noted.

19. In the well-conducted and documented repeated dose preclinical toxicity studies performed by intravenous (i.v.) route in dogs and monkeys, the signs of toxicity of DAS (named anguidine) were fairly consistent for both species on the various dosing schedules and included emesis, diarrhoea, erythema, increased haematocrit, anaemia, leukocytosis and/or leukopenia, neutrophilia, lymphopenia, elevated aspartate aminotransferase (AST) and alanine transaminase (ALT), elevated blood urea nitrogen (BUN) and nucleated erythrocytes. The lowest dose without toxic effect (NOAEL)

[4] Emesis: the action or process of vomiting.

regarding emesis and haematological adverse effects was equal to 0.016 mg/kg bw per day for dogs and to 0.125 mg/kg bw per day for monkeys.

20. The CONTAM Panel noted that adverse effects observed in preclinical studies performed by i.v. with DAS were similar to those described in acute toxicity studies after oral administration.

21. DAS as other trichothecenes are known to induce leukopenia, agranulocytosis, anaemia, aplastic anaemia due to cytotoxicity on circulating blood cells and on haematopoietic progenitors cells, the source of blood cells renewing in bone marrow (Parent-Massin, 2004; EFSA CONTAM Panel, 2011, 2017; JECFA, 2016).

22. No chronic studies have been identified in the literature.

23. The Panel considered that there are currently insufficient data on the genotoxicity of DAS. There was no evidence that DAS induces bacterial reverse mutation *in vitro*. One *in vivo* genotoxicity study in mice has been reported where DAS was administered by the i.p. route. Chromosomal abnormalities were observed in somatic cells (bone marrow) and in germ cells (spermatocytes). It is probable that the impairment of DNA synthesis is caused as a secondary event of inhibition of protein synthesis. Protein synthesis inhibition is likely to be the mechanism underlying the observed *in vivo* chromosomal abnormalities.

Human observations

24. The Panel identified data on adverse health effects in humans exposed to DAS in clinical studies and some epidemiological data which suggested an association with the incidence of 'alimentary toxic aleukia' (AKA) disease that possibly related to DAS. AKA was a human food poisoning outbreak occurring in the early 20th Century in the Soviet Union which has been associated with the consumption of food contaminated with *Fusarium* toxins, T-2 and particularly with DAS, having been suspected although not confirmed (Joffe, 1974; Joffe and Yagen, 1978; Bennett and Klich, 2003). The symptoms included inflammation of the GI tract in the early disease stages, followed by leukopenia, anaemia and other haematotoxicity symptoms, and lesions and necrosis in the mouth and gut. The CONTAM Panel noted that some of these symptoms have close similarity with symptoms observed as clinical side effects of DAS but the Panel considered the available data unsuitable for hazard characterisation of DAS in humans.

25. In the 1970s, DAS (named as 'anguidine') was developed as a potential anticancer drug and tested in a series of phase I and phase II trials on patients with different cancer types. Therefore, DAS has been evaluated as a candidate anticancer drug from the 1970s until mid-1980s, in both phase I and II studies (Tables 1 & 2). Exclusively given by i.v. administration, the established route for chemotherapy at these times, DAS doses ranged between 2.7 and 270 mg/kg bw/day (0.1-10 mg/m^2) in these studies, with

different treatment schedules *e.g.* i.v. injection (bolus) or infusion (up to several hours).

26. The CONTAM Panel considered the data on the toxicity of DAS collected in these clinical trials on about 500 patients as informative for the hazard characterisation of DAS.

27. Phase I and Phase II clinical trials showed similar toxicity at a dosing regimen of 5 mg/m^2 per day (or 3 mg/m^2 per day, if liver function was impaired). Doses of 3 mg DAS/m^2 day (81 µg/kg bw) and above are associated with substantial toxicities, particularly nausea and vomiting, myelosuppression, haematological toxicity and hypotension which can be life threatening at doses of 5 mg/m^2 per day (135 µg/kg bw) and higher. Although these phase II clinical trials confirmed DAS-related adverse effects, for the characteristics and objectives of the studies, they were considered of limited value for identifying reference points (RPs) for hazard characterisation.

Human hazard characterisation and derivation of health-based guidance values

28. Based on the conclusions on the genotoxicity and the mode of action of DAS the CONTAM Panel decided to establish HBGVs for both the acute and chronic exposure of DAS for humans

Table 1: Summary table for i.v clinical studies with DAS (anguidine) as an anticancer agent in Phase I trials.

	Dose						
Cancer (Organ)	**mg/m^2 per day**	**µg/kg bw per day [a]**	**Duration/ Interval Days**	**Route**	**N° of patients treated (N° patients evaluated)**	**Adverse effects (µg/kg bw/day or mg/m^2 per day)**	**Reference**
Advanced malignancies resistant to conventional therapeutic modalities	From 0.2 up to 6 [dose escalation]	From 5.4 up to 162	Daily for 5 days (one month interval)	i.v. From bolus up to 8 h infusion	36 (24)	Dose ≤ 2.4 nausea, vomiting Dose ≥ 2.4 neurotoxicity, GI toxicity, cardiotoxicity Myelotoxicity (inacceptable for highest dose)	Goodwin et al. (1978)
Advanced malignancies	From 0.1 and then 0.4 0.6–1 1.2–2.4 3–7.5 [dose escalation]	From 2.7 then 11 16–27 32–64 81–203	Daily for 5 days at 2-week intervals	i.v. 30–60 min Infusion	39 (33)	Dose ≥ 3, nausea, vomiting, hypotension, central nervous system symptoms (including somnolence, confusion, and ataxia), diarrhoea, chills and fever, generalised burning erythema, stomatitis, shortness of breath, moderate myelosuppression	Murphy et al. (1978)
Advanced malignancies resistant to conventional therapeutic modalities	2, 3.5, 4, 6, 6.5, 10 (weekly infusion) [dose escalation]	54, 94, 108,162, 175, 270	Until 36 weeks (5 patients)	i.v. 4–8 h Infusion	20	Dose ≥ 2 gastrointestinal and neurologic toxic effects. Thrombocytopenia at lowest doses (2–3.5)	Belt et al. (1979)
Gastrointestinal malignancies	From 1.5, then 3, 5, 7.5 (weekly infusion)	From 40 then 81, 135, 203	Repeated for 3–6 weeks	i.v. 3 h Infusion	29 (23)	Dose ≥ 3 Nausea and vomiting, hypotension, CNS symptoms (confusion, hallucinations, and	DeSimone et al. (1979)

	[dose escalation]					psychomotor seizures), chills, fever, and diarrhoea	

bw: body weight; i.v. intravenous
[a] Transformation factor of 37 (FDA, 2005) to convert the dose from m^2 to kg/bw

Table 2: Summary table for i.v. clinical studies with DAS (anguidine) as an anticancer agent in Phase II trials.

	Dose						
Cancer (Organ)	**mg/m^2 per day**	**µg/kg bw per day[a]**	**Duration/ Interval Days**	**Route**	**N° of patients treated (N° patients evaluated)**	**Adverse effects (µg/kg bw/day or mg/m^2 per day)**	**Reference**
Metastatic Adenocarcinoma colon rectum	3.5 – liver injuries – or 5	94, 135	Daily for 5 days at 3- week intervals	i.v. 2–3 h infusion	19	Hypotension (acute) Nausea and vomiting tolerable Myelosuppression moderate Fever and chills	Diggs et al. (1978)
Sarcoma	4.5	121	Daily for 5 days every 21 days.	i.v. 4-h infusion	27 (25)	Myelotoxicity, nausea and vomiting, mild to moderate hypotension	Thigpen et al. (1981)
Nervous system tumour	3.5–5 Weekly infusion	94, 135	with schedule	i.v. 4-h infusion	17 (16)	Myelosuppression, gastrointestinal symptoms, and central nervous system symptoms	Goodwin et al. (1983)
7 different types	3–5	94, 135	4- or 5-day continuous infusion every 28 days	i.v. Continuous infusion	276 (177)	Myelotoxicity	Adler et al. (1984)
Colorectal adenocarcinomas	5 mg/m^2 daily x 3 weeks	135	Daily for 5 days every 21 days.	i.v 3–6 h infusion	33 (29)	Hypotension (7/29), fever (7/29), CNS (3/29), myelotoxicity (3/29)	DeSimone et al. (1986)

bw: body weight; i.v. intravenous
[a] Transformation factor of 37 (FDA, 2005[5]) used to convert the dose from mg/m^2 to mg/kg bw

Human hazard characterisation

29. Human data from clinical trials (Tables 1 & 2) were considered by the CONTAM Panel as relevant, consistent and sufficiently informative to establish HBGVs for both acute and chronic exposure of humans. No clinical studies using oral administration of DAS and no other data in humans that would inform on the hazard of oral exposure were identified.

30. The CONTAM Panel decided to base the human hazard characterisation of DAS on the available clinical studies in patients treated by i.v. administration of DAS (anguidine) for cancer. Data on the toxicity of DAS was available for almost 500 patients recruited in phase I and II clinical trials

[5] Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers, FDA - Center for drug evaluation and research, July 2005.

conducted between 1978 and 1986. The Panel noted that about 100 patients were treated in clinical dose-finding studies of phase I starting with i.v. doses as low as about 0.1 mg DAS/m^2 (corresponding to 0.0027 mg DAS/kg bw) per day. These doses were chosen low enough to avoid signs of toxicity. Since the treatment period lasted from a few weeks to a few months the CONTAM Panel considered these clinical trials as short-term studies of DAS. The reported data on clinical side effects (also denoted as adverse effects or events in the context of clinical drug development) were considered as informative for oral toxicity of DAS in humans.

31. It was also noted that these patients may be considered as a specific vulnerable subpopulation since patients entering phase I studies usually are at advanced cancer stage and in general already pre-treated with other anticancer drugs.

Relationship between oral and i.v. administration and toxicity of DAS

32. In order to evaluate a relationship between the i.v. and the oral exposure of DAS, the CONTAM Panel assessed all available information pertaining to ADME characteristics in humans and in animals.

33. No pharmacokinetic data in humans were available from clinical studies after i.v. administration of DAS and no data after oral administration were identified either in humans or in experimental animals. Therefore, neither metabolic nor kinetic differences between oral and i.v. administration of DAS could be assessed for humans. In general, oral administration is expected to lead to delayed and lower peak concentrations in the organism, with longer half-life than after i.v. administration (Timbrell, 2000; Goodman et al., 2001).

34. In addition, after oral ingestion, DAS might be subject to gastro-intestinal degradation and a strong first-pass effect with early production of metabolites may occur. There were also indications from *in vitro* studies that DAS might be deepoxidated or deactylated to a large extent in the gut.

35. The CONTAM Panel considered that the available data from animals are suggestive for an almost complete absorption and/or quick clearance after oral and i.v. administration. Accounting for some variability in the bioavailability, the levels of DAS after oral administration may reach a fraction of up to about 80% of the i.v. levels. Therefore, considering exposure after oral dosing as equivalent to i.v. dosing can be regarded as a worst-case scenario. The limited available experimental data suggested that the toxicity of DAS when given orally was not higher than the toxicity after i.v. administration, as shown in the comparison of LD_{50} values over various species (Table 3).

Table 3: Summary table of acute toxicity data associated with oral, intravenous and intraperitoneal exposure in experimental animals.

Species	LD_{50} (mg/kg bw)		
	Oral	**Intravenous**	**Intraperitoneal**
Mouse		12 [a]	23 [b]
Mouse CD-I	15.5 [a] 12 [b]		20 [a]
Rat	7.3 [a]	1.3 [a]	0.75 [b]
Rabbit		1.0 [a]	
Guinea pig	2.14 [c]		
Dog		~1.1 [a]	

Key/Reference
[a] Conner et al (1986); [b] Ueno et al (1983)[*]; [c] Kriegleder et al (1981); bw: body weight
[*]LD_{50} values extracted from Stähelin et al (1968)

36. In humans, severity of adverse effects observed in clinical use of DAS was correlated with the duration of the i.v. infusion. Bolus injection (*e.g.* 5–10 min) caused more severe acute effects (mainly nausea, vomiting, hypotension and central nervous system (CNS) toxicity compared to prolonged infusion (*e.g.* 4–8 h) with a tendency to an increased severity of haematotoxicity and myelotoxicity.

37. The Panel assumed that the toxicity of DAS after oral administration would not lead to higher systemic toxicity compared to that after i.v. administration when taking into account the similar or lower toxicity of DAS metabolites.

38. The CONTAM Panel, however, noted data gaps that prevent a proper assessment of possible GI toxicity (*i.e.* mucositis) of DAS when orally ingested. Furthermore, the available clinical data of patients treated *via* i.v. do not permit a full assessment of local oral and GI tract toxicity. Oral administration might lead to exposure by contact in the upper GI tract. The Panel noted that GI effects, and particularly oral mucositis, are mainly ascribed to damage of basal mucosal layers after i.v. administration of anticancer cytotoxic chemotherapy (Duncan and Grant, 2003; van Vliet et al., 2010).

39. It is important to note, that in clinical trials with cytotoxic drugs mucositis often occurs together with other adverse effects such as GI symptoms, nausea and vomiting, haematotoxicity and CNS toxicity. It should be noted that the toxicity profile of DAS in phase II trials conducted at 3–5 mg/m^2 per day was very similar to the profile seen in phase II studies. The absence of effects in the mucosa in a total of 11 independent i.v. clinical studies of DAS would therefore not support the hypothesis that oral toxicity of DAS would be significantly larger than i.v. toxicity at similar doses.

40. Toxicity after oral exposure of DAS was considered as similar to, or lower than, that after i.v. administration and equitoxic effects for the same DAS dose were assumed when DAS was administered orally or intravenously. That might lead to an overestimation of the hazard after oral exposure such that the CONTAM Panel concluded that the use of data generated by i.v. administration might represent a worst-case scenario for

hazard characterisation and therefore be adequately protective for the consumers.

EFSA

Critical effects and derivation of an acute reference dose (ARfD) for DAS

41. Data on acute adverse health effects in humans were identified in clinical phase I studies when DAS was administered to humans in dose-finding studies.

42. The CONTAM Panel identified nausea and emesis as the most prominent acute adverse health effect of DAS in humans and selected this as the critical effect to determine a RP for the acute risk of humans exposed to DAS. The dose of 1.2 mg DAS/m^2, equivalent to 0.032 mg DAS/kg bw, was indicated as a dose without any emetic effect from clinical studies of phase I[6]. Conservatively assuming 100% bioavailability, this dose was used as the basis for setting an ARfD.

43. The CONTAM Panel considered these data sufficiently informative to determine a low level of dietary human intake at which no substantial acute health effects occurred.

44. To ensure a more protective approach, the Panel considered the application of an uncertainty factor (UF) for inter-human variability (EFSA, 2012) and used the UF of 10 to account for differences in toxicokinetics and toxicodynamics between humans. This is considered as a conservative approach since human variability and toxicokinetic differences would be limited to metabolism and excretion in the case of i.v. administration. Therefore, applying the UF of 10 to the RP of 0.032 mg DAS/kg bw identified above, the CONTAM Panel established 0.0032 mg DAS/kg bw (*i.e.* 3.2 µg DAS/kg bw) as ARfD for the acute exposure of humans to DAS.

Critical effects and derivation of a tolerable daily intake (TDI) for DAS

45. The CONTAM Panel identified haematotoxicity and myelotoxicity as critical adverse health effects after repeated exposure to DAS in clinical phase I studies as pivotal data for human chronic hazard characterisation. These observed adverse health effects are expected to induce leukopenia, agranulocytosis, and anaemia due to its cytotoxicity on circulating blood cells and on haematopoietic progenitor cells in bone marrow. The data from three prospectively defined dose-escalation schemes were investigated for the identification of a NOAEL for haematotoxicity and myelotoxicity (DeSimone et al., 1979; Murphy et al., 1978; Goodwin et al., 1978-Tables 1&2). The CONTAM Panel concluded from these data that haematotoxicity and

[6] The CONTAM Panel acknowledged that the cancer patients might represent a vulnerable population more prone to develop adverse acute effect (mainly nausea and vomiting) compared with the general population.

myelotoxicity would not constitute an adverse health effect in humans when exposed at a dose of 2.4 mg/m^2 per day (*i.e.* 65 µg/kg bw per day).

46. Phase II clinical trials confirmed DAS-related adverse effects, for the characteristics and objectives of the studies, however, they were considered of limited value for identifying a RP for hazard characterisation. To account for inter-human toxicokinetic (possible accumulation in bone marrow cannot be assessed or ruled out) and toxicodynamic variability of DAS, the CONTAM Panel applied the UF of 10 (EFSA, 2012) on this RP for chronic adverse health effects.

47. Considering the limited duration of the exposure, and the relatively limited number of patients exposed in the phase I studies used for the determination of the RP for chronic exposure above, the Panel decided to apply an additional UF of 10 to adjust for those limitations. This should be considered as a conservative approach. Therefore, the CONTAM Panel applied an overall UF of 100 to the NOAEL of 65 µg DAS/kg bw per day, and established a TDI of 0.65 µg/kg per day for the chronic dietary exposure of humans to DAS.

JECFA

48. JECFA (2017) concluded that there were insufficient toxicological data available to derive a point of departure (POD) for risk characterisation. There were also limitations in the available short-term toxicity studies and no data for chronic exposures or reproductive and developmental toxicity studies. (FAO/WHO, 2017).

49. 4,15-DAS is structurally similar to T2 and HT2 toxins with evidence that they cause similar effects at the biochemical and cellular levels, have similar effects *in vivo* and an additive dose when co-exposure occurs. Therefore, JECFA included 4,15-DAS in the group provisional maximum tolerable daily intake (PMTDI) for T2 and HT2, established by JECFA (FAO/WHO, 2001) of 0.06 µg/kg bw. This was based on a lowest observed adverse effect level (LOAEL) of 0.03 mg/kg bw/day associated with changes in white blood cell counts, following 3 weeks of dietary exposure in pigs and application of an uncertainty factor of 500 (FAO/WHO, 2017).

Exposure Assessment

50. Chronic 4,15-DAS exposures were calculated and are shown in Tables 1a-c. Acute 4,15-DAS exposures were also calculated and are shown in Tables 2a-c. Levels in the majority of food samples were below the limit of quantification (LOQ). Exposure assessments were expressed as a range of lower bound (LB) and upper bound (UB)[7]. Food groups that had values

[7] LB: (where 0 is used as the analytical value) UB: (the limit of detection/quantification is used).

between the limit of detection (LOD) and LOQ for 4,15-DAS included pasta, pizza, vegetable oils and dried pulses.

51. Tables 1a-c show mean and 97.5th percentile LB to UB chronic exposures for infants aged 4 to 12 months ranged from 0.00021 – 0.028 and 0.0013 – 0.092 µg/kg bw/day, respectively. For young children aged 12 to 18 months the LB-UB mean and 97.5th percentile chronic exposures ranged from 0.0015 – 0.038 and 0.0041 – 0.12 µg/kg bw/day. Mean and 97.5th percentile LB-UB dietary chronic exposures for young children aged 18 to 60 months ranged from 0.0017 – 0.033 and 0.0050 – 0.077 µg/kg bw/day.

52. Tables 2a-c show LB-UB Mean and 97.5th percentile acute exposures for infants aged 4 to 12 months ranged from 0.00050 – 0.055 and 0.0030 – 0.17 µg/kg bw/day, respectively. For young children aged 12 to 18 months the mean and 97.5th percentile acute exposures ranged from 0.0028 – 0.075 and 0.0077 – 0.21 µg/kg bw/day. Calculated mean and 97.5th percentile dietary acute exposures for young children aged 18 to 60 months ranged from 0.0023 – 0.077 and 0.0056 – 0.24 µg/kg bw/day.

53. The food groups which contributed most highly to total exposure in infants and young children were “potatoes” and “miscellaneous cereals - pasta”.

Table 1a. Estimated (LB-UB) 4,15-DAS chronic exposures from the total diet study (TDS) in infants aged 4 to 12 months (µg/kg bw/day)

4 to <6 month-olds (n=116)		**6 to <9 month-olds (n=606)**		**9 to <12 month-olds (n=686)**	
Mean	**97.5th percentile**	**Mean**	**97.5th percentile**	**Mean**	**97.5th percentile**
0.00021-0.00059	0.0013-0.036	0.00073-0.019	0.0033-0.085	0.0011-0.028	0.0038-0.092

Values rounded to 2 significant figures (SF)

Table 1b. Estimated 4,15-DAS chronic exposures from the TDS young children aged 12 to 18 months (µg/kg bw/day)

12 to <15 month-olds (n=670)		**15 to 18 month-olds (n=605)**	
Mean	**97.5th percentile**	**Mean**	**97.5th percentile**
0.0015-0.038	0.0046-0.12	0.0015-0.038	0.0041-0.098

Values rounded to 2 significant figures (SF)

Table 1c. Estimated 4,15-DAS chronic exposures from the TDS young children aged 18 to 60 months (µg/kg bw/day)

18 to 24 month-olds (n=158)		24 to 60 month-olds (n=978)	
Mean	**97.5th percentile**	**Mean**	**97.5th percentile**
0.0017-0.040	0.0050-0.12	0.0013-0.033	0.0031-0.077

Values rounded to 2 significant figures (SF)

Table 2a. Estimated 4,15-DAS acute exposures from the TDS in infants aged 4 to 12 months (µg/kg bw/day)

4 to <6 month-olds (n=116)		6 to <9 month-olds (n=606)		9 to <12 month-olds (n=686)	
Mean	**97.5th percentile**	**Mean**	**97.5th percentile**	**Mean**	**97.5th percentile**
0.00050-0.0014	0.0030-0.083	0.0016-0.041	0.0060-0.16	0.0021-0.055	0.0070-0.17

Values rounded to 2 significant figures (SF)

Table 2b. Estimated 4,15-DAS acute exposures from the TDS young children aged 12 to 18 months (µg/kg bw/day)

12 to <15 month-olds (n=670)		15 to 18 month-olds (n=605)	
Mean	**97.5th percentile**	**Mean**	**97.5th percentile**
0.0028-0.073	0.0081-0.21	0.0030-0.075	0.0077-0.19

Values rounded to 2 significant figures (SF)

Table 2c. Estimated 4,15-DAS acute exposures from the TDS young children aged 18 to 60 months (µg/kg bw/day)

18 to 24 month-olds (n=158)		24 to 60 month-olds (n=978)	
Mean	**97.5th percentile**	**Mean**	**97.5th percentile**
0.0032-0.077	0.0096-0.24	0.0023-0.060	0.0056-0.14

Values rounded to 2 significant figures (SF)

Risk characterisation

Exposures compared to JECFA PMTDI

54. Mean 4,15-DAS exposures for all age groups were below the JECFA PMTDI of 0.06 µg/kg bw/day.

55. The 97.5th percentile exposure for infants aged 4 to <6 months was also below the PMTDI. However, 97.5th percentile exposures for all other age groups exceeded the PMTDI of 0.06 µg/kg bw/day and ranged from 140 – 200 % of the PMTDI.

56. Some of the chronic exposures exceed the JECFA PMTDI of 0.06 µg/kg bw by 283% (2.8 fold), however, these are for the 97.5th percentile. If exposures to 4,15 DAS remained at this level for a prolonged period there could be some risk to health.

57. The JECFA HBGV is a group PMTDI for T2, HT2 and 4, 15-DAS. Therefore, with the addition of possible T2 and HT2 exposures it could be anticipated that a combined exposure from all 3 mycotoxins would further exceed the PMTDI. However, in their recommendations JECFA propose an update of the 2001 T2 and HT2 evaluation as the Committee had been made aware of new toxicity studies. (FAO/WHO, 2017).

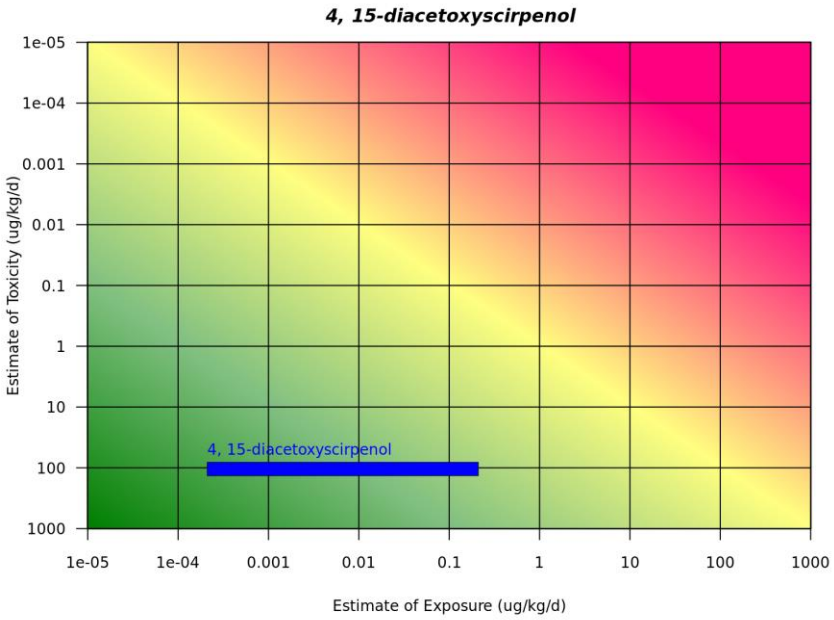
Exposures compared to EFSA ARfD and TDI

58. All the estimated mean and 97.5th percentile acute and chronic exposure levels were below the ARfD and TDI established by EFSA, respectively. The impact of the uncertainties in the human risk assessment of DAS is large and the risk is more likely to be over than underestimated. Some of the main uncertainties were the lack of co-exposure and potential combined effects of DAS and other trichothecenes and the lack of genotoxicity/carcinogenicity data.

RISK21

The RISK21 integrated evaluation strategy is a problem formulation-based exposure-driven risk assessment roadmap that takes advantage of existing information to graphically represent the intersection of exposure and toxicity data on a highly visual matrix.

Figure 1 shows a visual comparison of potential exposure and toxicity information using the mean and 97.5th percentile exposure for 4, 15-DAS in the diet of infants aged 0 to 12 months and children aged 1 to 5 years and toxicity information available. 4, 15-DAS sits in the green area (*i.e.* lower end of the exposure scenario) so a low risk.



Conclusions/Discussion

59. 4,15-DAS is a type A trichothecene mycotoxin produced by several *Fusarium* species. It has mainly been detected in cereal grains, cereal-based products and coffee but presence in other foods and feeds cannot be excluded.

60. From ADME studies, it was concluded that DAS is metabolised *in vitro* and *in vivo* to a large number of metabolites. The main metabolic processes are deacylations, hydroxylations, deepoxidations and glucuronide conjugations. Tissue concentrations decreased rapidly with no apparent accumulation in any tissues.

61. The CONTAM Panel identified adverse health effects in humans exposed to DAS when it was tested as a cytostatic anticancer drug (named anguidine) in phase I and phase II clinical trials on cancer patients by i.v. administration.

62. Based on the data of the phase I studies, the CONTAM Panel identified nausea and vomiting as the most relevant acute adverse health effects of DAS when administered i.v. with a NOAEL at 1.2 mg DAS/m^2 (equivalent to 32 µg DAS/kg)

63. Haematotoxicity and myelosuppression were the most frequently observed and persistent adverse effects observed in the phase I studies when DAS was given repeatedly (5-day regimen) in treatment cycles of 3–4 weeks. A NOAEL of 2.4 mg DAS/m^2 (equivalent to 65 µg DAS/kg bw per day) was identified from the same phase I studies.

64. The reported adverse health effects at doses from 3 to 5 mg DAS/m^2 (equivalent to 81-135 µg DAS/kg bw per day) of the phase II clinical trials,

performed at the proximity of the maximum tolerable doses, supported these findings.

65. Overall, EFSA established a TDI of 0.65 µg DAS/kg bw per day and an ARfD of 3.2 µg DAS/kg bw per day

66. JECFA set a PMTDI of 0.06 µg/kg bw, < 10 fold lower than the EFSA TDI.

67. Acute exposure estimates ranged for the mean from a minimum LB of 0.00050 µg/kg bw to a maximum UB of 0.077 µg/kg bw across different surveys and population groups (4-60 months old). The corresponding 97.5th percentile exposures, the values range from 0.0030 to 0.21 µg/kg bw.

68. Chronic exposure estimates ranged for the mean from a minimum LB of 0.00021 µg/kg bw to a maximum UB of 0.040 µg/kg bw across different surveys and population groups (4-60 months old). When considering the 97.5th percentile, the values were ranging from 0.0013 to 0.12 µg/kg bw.

69. All the estimated mean and 97.5th percentile acute and chronic exposure levels were below the ARfD and TDI established by EFSA, respectively, and as a result, not of health concern. However, the estimated mean and 97.5th percentile exceed the PMTDI by JECFA up to 200 % and 283%, respectively If exposures to 4,15 DAS remained at this level for a prolonged period there could be some risk to health.

70. <u>Questions to be asked of the Committee</u>

i). Do the Committee consider the epidemiology data conclusive enough to base the ARfD and TDI as the data is from cancer patients *i.e.* vulnerable groups?

ii). Do the Committee have any thoughts on the toxicity data when comparing i.v. vs oral exposure in relation to GI toxicity?

iii). Do the Committee have any other comments on this discussion paper?

iv). Do the Committee want a separates statement for DAS or can it be included in the overarching statement?

Secretariat

November 2018

Abbreviations

ADME	Absorption, Distribution, Metabolism and Excretion
ARfD	acute reference dose
ALT	alanine transaminase
AKA	alimentary toxic aleukia
AST	aspartate aminotransferase
bw	bodyweight
BUN	blood urea nitrogen
CCK	cholecystokinin
EFSA	European Food Safety Authority
DAS	diacetoxyscirpenol
FERA	Food and Environment Research Agency
GI	gastrointestinal
JECFA	Joint FAO/WHO Expert Committee on Food Additives
i.p.	intraperitoneal
i.v.	intravenous
HBGVs	health based guidance values
HT2	HT2 toxin
kg	kilogram
LD_{50}	lethal dose at which 50 % of the test population is dead
LOAEL	lowest observed adverse effect level
LOD	limit of detection
LOEL	lowest observed effect level
LOQ	limit of quantification
MAPKs	mitogen-activated protein kinases
µg	microgram
mg	milligram
NDNS	National Diet and Nutrition Survey
NOAEL	no-observed adverse effect level
NOEL	no-observed effect level
POD	point of departure
PMTDI	provisional maximum tolerable daily intake
RP	reference point
TDI	Tolerable Daily Intake
TDS	total diet study
T2	T2 toxin
UF	uncertainty factor
WHO	World Health Organization

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

Review of potential risks from 4, 15-diacetoxyscirpenol in the diet of infants aged 0 to 12 months and children aged 1 to 5 years

TOXICITY STUDIES DESCRIBED IN DETAIL

In vivo acute oral toxicity studies published and reviewed by EFSA (2018)

71. Conner et al. (1986) performed an acute toxicity study in male CD-I mice. DAS was administered to groups of four to six mice by gastric gavage with doses from 2 to 22 mg/kg bw, doubling the starting dose and later increasing it by a factor of 1.5. The LD_{50} at 96 h was calculated as 15.5 mg/kg bw. Lethal doses induced intestinal necrosis and extensive necrosis of lympho-haematopoietic organs. Sublethal doses of DAS induced cell depletion and necrosis in lympho-haematopoietic organs, multifocal necrosis of intestinal epithelium, and diffuse necrosis of germinal epithelium followed by progressive tubule degeneration in the testes. Leucocytosis, due to both lymphocytosis and neutrophilia in the first few hours after exposure to DAS, followed by lymphopenia, neutropenia and anaemia by 3 days were noted. There was rapid recovery of all sensitive organs after exposure to sublethal doses of DAS except for testis where decreased weights and abnormal spermatogenesis persisted for the 2-week observation period.

72. In order to investigate adverse effects in oesophagus and stomach, female Porton Wistar rats were fed DAS *via* oral route (gastric gavage) with single doses of 0.0125, 0.06, 0.125, 0.5 and 2.0 mg DAS/kg bw and the vehicle dimethyl sulfoxide (DMSO) alone as control (Craddock et al., 1988) with n=8 animals per group and sacrificing two rats per day on days 1−4, respectively, starving overnight before. An increase in cell replication 1 day after treatment in the oesophagus and in the squamous and glandular stomach was observed at the highest dose when compared with the control group. This effect increased further by day 2, and returned to normality by day 4. At the lowest dose, no increase in replication was observed in the oesophagus, but still in the squamous and glandular stomach at the doses from 0.06 to 0.5 mg/kg bw, with maximum increase after 1 day of treatment. At 0.06 mg/kg bw, the response was doubtful and at 0.0125 mg/kg bw staining of cells gave results similar to those of control animals.

In vivo acute intravenous toxicity studies published and reviewed by EFSA (2018)

73. DAS has been considered as a possible anticancer drug and it has been tested in preclinical studies by i.v. administration in dog and monkeys according to the supposed clinical schedule (5 consecutive days or weekly

administrations –IRDC report, 1973) to support the clinical development. In addition, DAS has been also tested by i.v. earlier on in rats, dogs and monkeys (Stähelin et al., 1968)

74. A preclinical study in support of anticancer drug development was performed in a total of 18 beagle dogs. One male and one female dog per group were exposed to a single injection of 0, 0.031, 0.063, 0.125, 0.25, 0.5, 1, 2 mg DAS/kg bw by i.v. (IRDC, 1973) with the exception of the group exposed to 1 mg DAS/kg bw where two males and two females were used. Complete haematological and biochemical investigations were performed every second day after treatment in the first week and weekly thereafter. One dog from each dosage level was sacrificed on day 8 (except at the 1 mg/kg dosage level, where two dogs were sacrificed) and the remaining dog(s) at each dosage level were sacrificed on day 45. At the highest dose, emesis, diarrhoea, slight tremors, licking of chops, injection of sclera and ataxia were noted for both dogs. At the lowest dose, no adverse effects compared to control were observed. Emesis, diarrhoea and haematological changes (e.g. neutropenia and lymphopenia) appeared from the dose of 0.063 mg/kg bw onwards and the frequency and severity of the toxicity was dose dependent. At the highest dose, the female dog was found dead 21 h after treatment. Moderate to marked increases in liver enzymes (*e.g.* alanine transaminase (ALT) and aspartate aminotransferase (AST)), haematocrit and nucleated erythrocyte count, leucocytosis, neutrophilia with increase in non-segmented neutrophils, blood urea nitrogen (BUN) were recorded. Lymphopenia and thrombocytopenia were noted in the male dog. All values had essentially returned to normal by day 8 of study for this dog. According to the authors, the dose without adverse effect (no-observed-adverse-effect level (NOAEL)) was 0.031 mg/kg bw. Coppock et al. (1989) performed a study on dogs. A total of eight dogs (four animals per dose group) were exposed to the vehicle or 0.5 mg/kg bw of DAS (purity> 98%) i.v. The animals were euthanatised at 8 h after treatment. Blood samples were taken at half-hour intervals. Sequential clinical signs of intoxication observed in the treatment dogs included ptyalism (hypersalivation), emesis, diarrhoea, ataxia, muscular weakness and depression. Histopathological investigation revealed lesions in the bone marrow of treatment animals consisting of cellular necrosis in the haematopoietic districts. A marked increase in the number of immature neutrophils and replacement of lymphocytes with immature cells was observed.

Repeated dose studies oral administration

75. Guinea pigs (4 animals per group) received DAS by drinking water, over 30 days in daily doses of 0,0.6, 1, 1.3 and 1.6 mg/kg bw (Kriegleder, 1980, 1981). With exception of the highest dose, feed intake and body weight gain were not significantly different from controls, although reduction in body weight gain was observed (up to 20%). All animals died in the highest dose group within 10 days and one animal per group died at the other dose levels (accidental death). Weekly haematological investigations were performed and bone marrow smears taken at the end of observation period. The authors

reported emesis, loss of appetite and reduced movement after the second day of treatment at the highest dose. An episode of lips necrosis was noted. In the animals that died from day 5 onwards histological signs of necrosis in GI tract, lymph nodes and bone marrow were noted. No significant changes in haematological parameters (n=8 animals evaluable in the three dose groups) were noted.

Repeated dose studies intravenous administration

76. The CONTAM Panel noted that adverse effects observed in preclinical studies performed by i.v. with DAS were similar to those described in acute toxicity studies after oral administration. DAS as other trichothecenes are known to induce leukopenia, agranulocytosis, anaemia, aplastic anaemia due to cytotoxicity on circulating blood cells and on haematopoietic progenitors cells, the source of blood cells renewing in bone marrow (Parent-Massin, 2004; EFSA CONTAM Panel, 2011, 2017; JECFA, 2016).

77. Rats (10 males and 10 females per group) were exposed via i.v. for 4 weeks to 0.06, 0.18 and 0.54mg DAS/kg bw per day (Stähelin et al., 1968). Mortality was observed at low dose (single death) and up to 75% of rats in the high-dose groups. Adverse effects observed with dose-relationship included weight reduction, diarrhoea, haematuria, reduced testis and liver weight. Histopathological examination showed findings in the liver (necrotic foci, bile duct and reticuloendothelial cells proliferation), in spleen (follicle hypoplasia) and testis (tubule atrophy and blocked spermatogenesis). Decrease in leucocytes count and minor reduction in erythrocytes count have been observed after 4 weeks starting from animals exposed to the middle dose and with dose dependency. Effects in bone marrow have been noted only at high dose level (e.g. decrease of granulopoiesis). Six rats were additionally treated by i.v. for 6 weeks with doses increasing during the study from 0.15 up to 0.5 mg DAS/kg bw with treatment-free periods−no further details available (Stähelin et al.,1968). No mortality was recorded and only haematological effects (e.g. variable degrees of leukopenia and lymphopenia) were observed.

78. In a study performed by Stäahelin et al. (1968), four dogs per group were treated via i.v. daily for 4weeks with 0, 0.02, 0.06 and 0.18 mg DAS/kg bw per day. At the highest dose, three dogs died during the treatment period and vomiting after each administration, bloody stool and weight loss was recorded. No adverse effects were observed at the lowest dose. A decrease in body weight was noted from 0.06 mg/kg bw day. According to the authors, no haematological changes were observed at the lowest dose, while lymphopenia and erythroblastosis in bone marrow appeared from the middle dose. In the survived dog at the highest dose, leukopenia, follicular hyperplasia in spleen, reduction of number of haematopoietic cells in bone marrow were noted. At low doses, it was histologically observed reduction of lymphatic tissue in spleen, proliferation of reticuloendothelial cells and degenerated in follicle germ cells, atrophy and reduced maturation in testis and pyknotic cells in intestinal epithelium. Overall, the CONTAM Panel concluded that in this study the lowest dose of 0.02mg DAS/kg bw can be considered as a NOAEL.

79. Daily treatment by i.v was carried out in two beagles (1 male and 1 female/group) during 5 days at dose equal to 0, 0.016, 0.031, 0.063, 0.125 and 0.25 mg/kg bw per day in a preclinical study (IRDC report, 1973) in support to anticancer drug development. Haematological investigations and liver function tests were periodically performed. One dog from each dosage level was sacrificed on day 12 and the remaining dog at each dosage level was sacrificed around day 50 of the observation period of the study. At the lowest dose, no adverse effects compared to control were observed. From 0.031 mg/kg bw per day, the presence of nucleated erythrocytes at haematology together with neutrophilia, lymphopenia (11% and 37% lymphocyte in male and female respectively compared to normal values 20–52%) and signs of slight anaemia, were noted for both dogs. Erythema and emesis after dosing were sporadically noted in dogs exposed to 0.063 mg/kg bw per day. Haematological troubles increased with the increase in the doses. According to the authors, the dose without toxic effect was the lowest dose tested, 0.0164,15-mg/kg b mg/kg bw per day. Overall, the CONTAM Panel concluded that in this study the lowest dose of 0.016 and 0.031 mg DAS/kg bw can be considered as a NOAEL for haematotoxicity and emesis, respectively.

80. Daily treatment by i.v. was performed in beagle dogs (1 male and 1 female/group) during 5 days followed by 9 days of rest, the treatment was repeated three times (IRDC report, 1973). The doses tested were 0.031 and 0.125 mg/kg bw per day. Complete haematological and biochemical investigations were periodically performed. One dog from each dose level was sacrificed on day 40 of the study and the remaining dog was sacrificed on day 78 at the end of observation period. Emesis was noted at the high dose. At laboratory examinations slight increases in leucocytes, neutrophils, eosinophils and hepatic enzymes (ALT, AST) together with decreased haematocrit were recorded. At the highest dose instead, haematological effects (namely leukopenia, neutropenia, relative lymphocytosis, thrombocytopenia, polychromasia of erythrocytes and slight anaemia) were noted with different severity in both dogs. Slight elevation in hepatic enzymes was also recorded during treatment periods. Overall, the CONTAM Panel concluded that in this study the lowest dose of 0.031 mg DAS/kg bw can be considered as a NOAEL for emesis. Weekly treatment by i.v. was also performed in beagle dogs (2 animals per group) during 6 weeks at doses of 0.031, 0.063, 0.125 and 0.25 mg/kg bw (IRDC report, 1973). Haematological and biochemical investigations were regularly performed during the treatment period. One dog from each dosage level was sacrificed on day 43 of study and the remaining dog was sacrificed on day 81 of study, at the end of the observation period. At the lowest dose, the only effects noted were slight and sporadic and consisted in anaemia, leuco- and thrombocytopenia, reticulocyteosis and elevation of liver enzymes. From 0.063 mg/kg bw per week, emesis and slight erythema post-dosing were frequently noted for the male dog and occasionally for the female dog. More marked anaemia, neutropenia and lymphopenia were noted at laboratory investigations with some dose. Overall, the CONTAM Panel concluded that also in this study the

lowest dose of 0.031 mg DAS/kg bw can be considered as a NOAEL for emesis.

81. DAS has been injected daily intravenously to rhesus monkeys (1 male and 1 female/group) during five consecutive days at doses equal to 0, 0.125, 0.25, 0.50 and 1.0 mg/kg bw per day in a preclinical study (IRDC report, 1973) for anticancer drug development. Complete haematological and biochemical investigations were carried out at regular intervals. One monkey from each dosage level was sacrificed on day 12 and the remaining monkey at each dosage level was sacrificed on day 50 of study. At the lowest dose (0.125 mg/kg bw per day), no adverse effects compared to control were identified. At the dose of 0.25 mg/kg bw per day, during the treatment period, emesis, anorexia, soft stool or diarrhoea and hypoactivity were noted. During and immediately after the 5-day of treatment, a decrease in the absolute number of leucocytes (up to 70% vs basal values) with lymphopenia and neutropenia was recorded. A change in leucocyte formula was also noted with neutrophils reaching the highest value of 85% (standard values ranging from 7% to 47%) and lymphocytes the lowest value of 14% (with a standard value ranging from 52% to 92%) with similar trend in both animals. At the dose of 0.5 mg/kg bw per day, both monkeys died on day 4. Death was preceded by hypothermia, anorexia, emesis and hypoactivity. At laboratory examinations leucocytosis (twice the basal values) with marked neutrophilia (>90% of the total leucocytes) was observed in both monkeys. At the highest dose (1 mg/kg bw per day), both monkeys died on day 3. At laboratory, examinations similar but more marked changes in comparison with the dose of 0.5 mg/kg bw per day were recorded for both monkeys (with leucocytes showing values three times the basal values and marked neutrophilia,>90% of the total leucocytes). Slight increases in some biochemical parameters (namely ALT, AST and BUN) before the death of animals were recorded. The CONTAM Panel noted that under the experimental conditions applied in this study the lowest dose tested, 0.125 mg/kg bw per day, can be considered as a NOAEL mg/kg bw per day. Overall, the CONTAM Panel concluded that in this study the lowest dose of 0.016 and 0.031 mg DAS/kg bw can be considered as a NOAEL for haematotoxicity and emesis, respectively.

82. Daily treatment by i.v. was performed in beagle dogs (1 male and 1 female/group) during 5 days followed by 9 days of rest, the treatment was repeated three times (IRDC report, 1973). The doses tested were 0.031 and 0.125 mg/kg bw per day. Complete haematological and biochemical investigations were periodically performed. One dog from each dose level was sacrificed on day 40 of the study and the remaining dog was sacrificed on day 78 at the end of observation period. Emesis was noted at the high dose. At laboratory examinations slight increases in leucocytes, neutrophils, eosinophils and hepatic enzymes (ALT, AST) together with decreased haematocrit were recorded. At the highest dose instead, haematological effects (namely leukopenia, neutropenia, relative lymphocytosis, thrombocytopenia, polychromasia of erythrocytes and slight anaemia) were noted with different severity in both dogs. Slight elevation in hepatic enzymes was also recorded during treatment periods. Overall, the CONTAM Panel

concluded that in this study the lowest dose of 0.031 mg DAS/kg bw can be considered as a NOAEL for emesis.

83. Weekly treatment by i.v. was also performed in beagle dogs (2 animals per group) during 6 weeks at doses of 0.031, 0.063, 0.125 and 0.25 mg/kg bw (IRDC report, 1973). Haematological and biochemical investigations were regularly performed during the treatment period. One dog from each dosage level was sacrificed on day 43 of study and the remaining dog was sacrificed on day 81 of study, at the end of the observation period. At the lowest dose, the only effects noted were slight and sporadic and consisted in anaemia, leuco- and thrombocytopenia, reticulocyteosis and elevation of liver enzymes. From 0.063 mg/kg bw per week, emesis and slight erythema post-dosing were frequently noted for the male dog and occasionally for the female dog. More marked anaemia, neutropenia and lymphopenia were noted at laboratory investigations with some dose. Overall, the CONTAM Panel concluded that also in this study the lowest dose of 0.031 mg DAS/kg bw can be considered as a NOAEL for emesis.

Chronic toxicity studies

Developmental and reproductive toxicity studies

84. The studies that have been reported on the developmental and reproductive toxicology of DAS all used i.p. administration of the compound. DAS was injected i.p. to male mice at doses of 1, 5, 10 or 15 mg/kg bw and animals were killed 1h to 14 days later. Testicular weights were decreased 3 days after DAS exposure (15 mg/kg bw) and this effect persisted throughout the observation period of 14 days. There was progressive depletion of germinal epithelium which was followed by tubule degeneration (Conner et al., 1986). Conner et al. (1990) investigated the testicular function in male Lewis rats that had been exposed to DAS at 1.7 mg/kg bw by i.p. injection. This dose is 75% of the i.p. LD_{50} as measured in the authors' laboratory. The animals were studied up to 90 days after exposure. DAS induced a decrease of the testicular weight and sperm production and an increased frequency of hypocellular seminiferous tubules. These effects became more marked with increasing time after administration of DAS. Pregnant mice were administered a single i.p. dose of DAS at 1.0, 1.5, 2.0, 3.0 and 6.0 mg/kg bw on one of gestation days 7–11 (Mayura et al., 1987). The two highest doses resulted in maternal toxicity. There was no significant effect on total number of implants at all dose levels tested during the various gestation periods. The incidence of resorption was dependent on dose, reaching 100% at the higher doses, and increases were seen at the lower doses. The exposure caused a reduction in fetal body weight even at the dose of 1.0 mg/kg bw, on all the days tested. Significant incidences of gross and skeletal malformations in mouse foetuses were observed at all doses of DAS tested (1–3 mg/kg bw). The authors commented that DAS is a potent inhibitor of protein synthesis and that this may be responsible for the effects induced by it.

85. In summary, the lowest dose where developmental and reproductive effects were seen in mice was 1 mg/kg bw (females, incidence of resorption, reduction in fetal body weight and gross and skeletal malformations), and in rats it was 1.7 mg/kg bw (males, decrease of the testicular weight and sperm production and an increased frequency of hypocellular seminiferous tubules). No lower doses were used and thus no NOAELs were observed. The CONTAM Panel noted that tissues with high proliferation rate such as testicular are targets of adverse effect of DAS.

Genotoxicity

in vitro studies

86. Wehner et al. (1978) showed that DAS did not induce mutation in the *Salmonella Typhimurium* bacterial mutation assay (Ames test) using strains TA98, TA100, TA1535 and TA1537 (0.25–250 µg DAS/plate) with and without metabolic activation with an induced rat liver S9 fraction. Kuczuk et al. (1978) obtained negative data in the *S.Typhimurium* bacterial mutation assay (Ames test) using strains TA1535 and TA1537 and TA1538 (0.1–100 µg DAS/plate) with and without metabolic activation with induced rat liver S9 fraction. DAS was also screened for mutagenic activity in *Saccharomyces cerevisiae* D-3 and none was detected: DAS was tested in this study at the concentration 100 µg/mL without metabolic activation, and at 50 µg/mL with metabolic activation.

87. Sinsheimer et al. (1989) found that DAS lacked alkylating activity as measured by its reaction with 4-(4-nitrobenzyl) pyridine, and also lacked mutagenicity using *S.Typhimurium* strain TA 100 (0.01–15 µmol DAS/plate).The genotoxicity of DAS *in Escherichia coli* was investigated by Krivobok et al. (1987) using the SOS chromo test, which is an assay to detect the SOS response. No SOS inducing activity was detected, either with or without metabolic activation. The maximal concentration of DAS that was tested was 60lg/ml. Cooray (1984) studied the effect of DAS on DNA synthesis, by measurement of [3H]-thymidine incorporation, and sister chromatid exchange (SCE) frequency in phytohemagglutinin-stimulated human peripheral lymphocytes. Addition of DAS to the cell cultures resulted in a dose-related inhibition of [3H]-thymidine incorporation, with complete inhibition at a concentration of 8 ng DAS/mL. Toxicity was slightly reduced by addition of rat liver cells to the incubation. No increase in SCE frequency was observed either with or without the presence of rat liver cells (1.5–12.0 ng DAS/mL).

in vivo studies

88. The genotoxicity of DAS following i.p. injection was investigated in male Swiss albino mice by Hassanane et al. (2000). For both single and repeat dose studies, groups of mice (n=5) received 0,0.5, 0.75 and 1 mg DAS/kg bw. In the repeat dose study, the dose was administered on days 1, 10and 20. The study was carried out on both somatic and germ cells. After single doses, DAS significantly reduced the mitotic activity of the bone marrow

cells at all doses tested. Increased structural chromosome abnormalities (chromatid gaps, breaks, centromeric attenuation, endomitosis) were observed after single doses. The increases of centromeric attenuation, endomitosis and total abnormalities were highly significant ($p<0.01$) for the two highest doses of DAS. After repeat doses, increased chromatid gaps, breaks and endomitosis were also observed. Finally, there was evidence of chromosome damage in spermatocytes, and sperm abnormality after DAS treatment. DAS gave negative results in the wing somatic mutation and recombination test (SMART) in *Drosophila melanogaster*, at concentrations ranging from 5 to 40lM (Gürbüzel et al., 2015).

Carcinogenicity studies

89. Lindenfelser et al. (1974) carried out a study on the initiating and promoting activity of mycotoxins in a 22-week skin tumour test according to Boutwell (1964) with a focus on the interactions of aflatoxin B1 with T-2 toxin and DAS. Groups of Charles River female mice (n=8) were treated, by skin application, with the initiating compound and then after 4 days promoter compounds were administered twice weekly for 22 weeks. The compounds that were tested for either initiation or promotions were aflatoxin B1, T-2 toxin and DAS, and positive controls were 7,12-dimethylbenz[a]anthracene (DMBA) (initiation) and croton oil (promotion). A variety of compound combinations were studied, and three of these involved DAS. These were: DMBA initiation (50 µg) with DAS promotion (10 and 25 µg), aflatoxin B1 initiation (25, 50 or 100 µg) with DAS promotion (10 and 25 µg) and DAS initiation (25 µg) with DAS promotion (10 µg). In addition, T-2 ability to act as initiator (25 µg) or promoter (10 and 25 µg) was also investigated. After DMBA initiation and DAS promotion there was a minimal tumour response (1 papilloma in a single mouse). Similarly, after DMBA initiation, one of the eight mice treated with T-2 developed one papilloma. No papillomas were seen in mice after aflatoxin B1 initiation and DAS or T-2 promotion. The group administered DAS (or T-2) over both the initiation and promotion stages developed no papillomas. After DMBA initiation and promotion with the positive control croton oil (1,000 µg) all eight mice had papillomas. After aflatoxin B1 initiation (25, 50 and 100 µg) and croton oil promotion, papillomas were observed in 5, 5 and 6 out of 8 mice, respectively. The authors concluded that there is an indication of weak promoting activity of DAS.

90. In summary, no long-term studies on carcinogenicity have been identified to assess carcinogenicity of DAS. No initiating activity was identified for DAS.

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