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Derivation of safe exposure levels for potential migration of formaldehyde into food

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ABSTRACT

Polyoxymethylene (POM) is a polymer of formaldehyde used inter alia for kitchenware and food processing machines. By migration into food, consumers may be exposed to small additional amounts of formaldehyde in food. In order to address such potential exposures, Specific Migration Limits are derived using all studies with oral exposure in mammals and birds. The assessment is not only based on local irritation observed in a 2-year rat study that has previously served to calculate acceptable exposure levels, but also on systemic effects, namely on effects on the kidney in adult rats and testes in birds before sexual maturity. At the relatively high oral exposure levels (up to 2000 ppm in drinking water) long-term effects caused by formic acid, the first step metabolite of formaldehyde, such as acidosis, cannot be excluded. The lowest Specific Migration Limit of 2.74 mg/dm^2, corresponding to 16.5 mg formaldehyde/kg food, is based upon kidney effects in rats, leading to potential exposures that range between 2900 and 4400 times below the endogenous turnover of formaldehyde. Lastly, a recent migration study with POM showed that migration of formaldehyde into food simulants is over an order of magnitude below the lowest Specific Migration Limit derived herein.

1. Introduction

Polyacetal or polyoxymethylene (POM) is a polymer popularized by its desirable mechanical properties and its ability to be used in many different hard plastic applications. An important group of products with food contact are kitchenware and food processing machines for multiple use that are made with POM, and these product applications are the focus of the present paper. In the majority of these so-called repeated use applications, the components and parts made of POM have only a limited surface area with food contact. Within this paper, the standard EU surface contact area is applied (6 dm^2) and consumption of 1 kg food product per day is assumed. These assumptions provide a conservative estimate of potential exposure and actual contact area and amounts of food consumed after contact with POM are likely to lead to much lower exposures of consumers.

POM is the stabilized linear polymer of formaldehyde (FA), but it may release trace amounts of FA into food or water and thereby lead to exposure of consumers to FA. FA has been evaluated by the Risk Assessment Committee (RAC, 2012) of ECHA leading to the classification Category 1 B for carcinogenicity (may cause cancer) and Category 2 for mutagenicity (suspected of causing genetic defects) (https://echa.europa.eu/de/information-on-chemicals/cl-inventory-database/-/discli/details). Although the strongest data are related only to local tumor induction in the nose after inhalation, the classification for carcinogenicity was not restricted to the inhalation route (i.e., H350i: may cause cancer by inhalation). With this classification it is necessary and timely to assess any potential risk to the human population of residual FA in food, via migration from food contact materials, and to establish a FA Specific Migration Limit (SML).

Exposure limits for the general population by oral ingestion have already been derived by a variety of regulatory and scientific organizations. These assessments are generally based on local irritation observed in the stomach of rats in the study of Til et al. (1989) described in more detail below. WHO (2005, 2011) proposed a tolerable concentration in drinking water of 2.6 mg/l derived from the NOAEL of 260 mg/l (Til et al., 1989) and a combined Uncertainty Factor (UF) of 100 for inter- and intra-species variability. The same conclusion was reached by CICAD (2002) (Concise International Chemical Assessment). The EU by Commission Regulation (EU) No 10/2011 (EU, 2011) defined a total SML(T) of 15 mg/kg food that also refers to FA releasing

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substances potentially in contact with food like hexamethylenetetramine (EU, 2011) or 1,4-butanediol formal that may hydrolyze to ammonia or butanediol and FA (EU, 2015), respectively.

With the EU classification of FA the question came up whether the SML of EU (2011) can still be maintained on a scientific basis. Therefore, a SML is derived here based on the NOAELs as Point of Departure (PoD) of all oral exposure studies available. Not only local irritation is taken into consideration as used for former derivation of safe exposure levels, but also potential systemic effects observed in experimental mammals and birds. After short sections with oral studies not used for derivation of an SML and on the mode of action identified by inhalation studies, the paper is organized according to the different toxicological endpoints: local irritation in repeated dose oral studies separately for mammals and birds, reproductive and developmental toxicity, immunotoxicity, neurotoxicity, other systemic endpoints, and genotoxicity. The question is discussed how FA, despite its rapid metabolic detoxification, might lead to systemic effects after high oral exposure. Formic acid, the first-step metabolite, is identified as potential toxic agent that may lead to sustained metabolic acidosis. SMLs are derived by the UFs of EFSA (2012) for the critical endpoints identified, namely local irritation, effects on the kidney in adult rats and gonads (and leukocytes) in juvenile birds. Finally, the SMLs were compared to internal production of FA and its external exposure via normal food constituents and migration into food simulants from POM.

Literature is taken from RAC (2012) (Risk Assessment Committee of ECHA, the European Chemicals Agency) and the International Uniform Chemical Information Database (IUCLID) submitted to ECHA (2017) under the REACH regulation (Registration, Evaluation, Authorisation and Restriction of Chemicals) by the Formaldehyde Consortium, with a last update from October 2015 (https://echa.europa.eu/registration-dossier/-/registered-dossier/15858). The assessments of CICAD (2002), WHO (2005) (World Health Organization) and BfR (2006) (Bundesinstitut für Risikobewertung, Germany) have also been taken into consideration. In addition, to ensure a comprehensive assessment, a literature search was carried out up to April 2019 to identify toxicity studies with oral exposure (key identifier oral, peroral, and gavage) that were cited in the following databases: CHEMLIST, REGISTRY, EMBASE and TOXCENTER.

2. Oral studies not used for derivation of SML

The livestock studies with oral exposure published by Nitsan and Bruckental (1977), Spears et al. (1980), Oldham et al. (1982), Schutte and Smith (1991), and Deniz et al. (1993) are not used because insufficient details were reported or the exposures to FA could not be estimated.

Preston et al. (1960) treated skim milk (1000 ml) with formalin (1 ml, 40% FA, corresponding to 400 mg/l milk) and exposed calves to untreated or FA treated skim milk over 3 d (3 calves/group). At necropsy macroscopic inspection and histopathology showed intensive inflammation of the abomasum and alterations extended down to the small intestine. This study confirmed local irritation of FA at the concentration used, but as the body weights of the calves and the daily or total consumption of the treated skim milk are not given, the exposure to FA related to body weight cannot be calculated.

Müller et al. (1978) developed an “oral tank” made of plastic material used for dental prosthetics that was fitted to the mucosa in the vestibulum oris of rabbits. The tank contained a sponge filled with 3% formalin being in contact with the mucosa by some holes. Under the assumption that formalin with 37% FA was used, the FA concentration in the tank was 11,100 mg/l. Six rabbits were used for exposure to FA and 4 rabbits as controls to ascertain potential irritation of the device itself. Exposure lasted 90 min/d, 5 times/week over 10 months. One month later the animals were sacrificed to study the oral mucosa by histopathology. Due to mechanical irritation the tank without FA led to moderate reactive hyperplasia with parakeratosis, while FA exposure (300 h in total) resulted in severe lesions of the oral mucosa with characteristics of a carcinoma in situ. While this study indicates that repeated exposure to high concentrations of FA may lead to neoplastic alterations in the oral cavity, the highly artificial setup of this experimental approach renders it unsuited for risk assessment purposes.

Bhatt and Panchal (1992) administered FA (source not mentioned) over 60 days to adult male rats by the oral (10,000 mg/l drinking water) or intraperitoneal route (10 mg/kg bw). Before exposure rats were trained to avoid electric shocks after a buzzer signal by climbing a wooden rod. Conditioned and unconditioned avoidance responses were recorded. The description of the experiment is very poor and the results are difficult to interpret. According to the authors, FA after intraperitoneal and oral application influenced memory. As regards oral exposure, the very high daily dose is noted. No effects were observed on body weight, posture or muscular activity. Taking into account that only one oral dose was used (not enabling dose-response assessments), the very high dose level and the poor description of methods and results, this study will not be taken into consideration.

There are several relatively new oral gavage studies the interpretation of which is hampered by several important drawbacks: detailed information is missing on the source of FA and dosing solution, histopathology of the stomach, the primary portal of entry, was not carried out, and only one dose level of FA was used such that a dose response relationship cannot be evaluated. Formalin was administered, presumably stabilized with methanol but no efforts were made to assess potential confounding of toxicity by methanol. Furthermore, the relatively steep rise in blood levels obtained by gavage leads to some uncertainty in risk assessment for humans exposed via food or liquid intake. Finally, the authors generally failed to discuss their observations in relation to other prominent studies in rats or mice, like e.g. the chronic study of Til et al. (1989) or the prenatal toxicity study of Marks et al. (1980). Therefore, the findings of these studies will only be summarized without going into details. These studies concentrated on effects by combined exposure to FA and other substances and here we will only report on the parts dealing solely with FA.

Soni et al. (2013) described necrosis of the liver after daily exposure of male mice to 25 mg FA/kg bw/d over 8 weeks. The strain of mice and their number per group are not given nor the FA concentrations or the dosing volume. The percentages of necrotic cells in the liver was extremely high with 14% in controls and 50% in treated groups while no health effects or deaths were reported. After reviewing the photographs given in the publication, a pathologist could not confirm hepatocellular necrosis but identified focal infiltrates, most probably of lymphoid cells. In addition, “necrotic” foci were increased by FA treatment but no information is available whether this relates to the number or area of the foci. Abd-Elhakim et al. (2016) observed in male Swiss mice alterations in the hematogram, leukogram and in immunological parameters at the same dose of FA over 60 consecutive days. FA treatment led to a 10% decrease of body weight. A 40% solution of FA (possibly formalin without further characterization) was diluted in water to “working stock solutions” without giving any details. Taking into account the dosing volume of 0.1 ml/mouse (20–25 g initial weight) a FA concentration in the range of 6000 mg/l can be estimated being by a factor of ~3 higher than the highest drinking water concentration used by Til et al. (1989). Under consideration of the bolus application of Abd-Elhakim et al. (2016) severe effects on the stomach are to be expected with unknown systemic sequelae and missing data in this respect is a severe deficiency. Khalil et al. (2017) from the same university obviously used the same approach for male Swiss mice with 25 mg FA/kg bw/d over 65 consecutive days. Again 40% FA was diluted to “working stock concentrations” without further details. The dosing volume per mouse was not specified but most probably corresponded to that of Abd-Elhakim et al. (2016). FA exposure affected steroidogenesis and several testicular enzymes and led to a 15% decrease of body weight. The same criticism applies as for Abd-Elhakim et al. (2016). Mohamed et al. (2017) observed in Dawley Albino rats (3 months of age) adverse

effects on biochemical liver and kidney parameters and histopathological alterations in these organs. The animals received 100 mg formalin/kg bw/d (concentration of FA not given) over 30 consecutive days. For a 40% formalin solution the dose would be 40 mg FA/kg bw/d. As the dosing volume was not specified the concentration of FA cannot be calculated. The number of rats used or the housing conditions were not specified. No summary or grading of histopathological lesions was provided and the figures and the text do not correspond to each other. Although the animals were weighed and observed for clinical signs no data is presented. Merzoug and Toumi (2017) observed alterations of behavior and hemato-immune parameters in pregnant Wistar rats with significantly increased cortisol and decreased 17β-estradiol. Rats were treated over 10 days before mating and then until gd 19. They were dosed with 2 mg FA/kg bw/d (from 37% formalin) administered in a volume of 1 ml/kg bw corresponding to 2000 mg/l similar to the highest concentration used by Til et al. (1989). Offspring showed a significant decrease of body weight, of several growth related parameters and especially a nearly 90% reduction of live fetuses. Again, under consideration of the bolus application severe effects in the stomach are to be expected but no information is available. The animals received only one dose clearly above the MTD, as the dams only had a body weight gain at 38% of controls and therefore all observations could result from a simple overdosing and be secondary to general toxicity.

The various deficiencies in the documentation of important technical details in all these publications do not allow an evaluation whether the effects observed are direct and specific effects of FA itself (which are at least in part not consistent with the existing extensive data base) or rather non-specific secondary effects of highly irritating bolus doses to the gastrointestinal tract. Thus, the results of these studies are not listed in the tables and will not be used for calculation of a SML for human exposure via food or liquid intake.

3. The mode of action (MoA) for carcinogenicity

RAC (2012) classified FA as a cat 1 B carcinogen based on nasal tumor formation in rodents after inhalation. For derivation of any exposure limit, the MoA is pivotal and the question needs to be answered whether the nasal tumors in experimental animals can be considered a threshold effect for which a NOAEL may be established. McGregor et al. (2006) showed that nasal carcinogenicity is driven by sustained cytotoxic irritation and cell proliferation with a clear threshold. The same conclusion was reached by WHO (2010) when developing their guideline for Indoor Air Quality and by Nielsen et al. (2010, 2013, 2016), who also evaluated the inconclusive epidemiological evidence for leukemogenic potential of formaldehyde and determined that no further adjustment to the WHO guideline value was warranted (Nielsen et al., 2016). SCOEL, (2016) considered FA as a group C carcinogen (genotoxic carcinogens for which a practical threshold is supported) according to the SCOEL (2013) guideline. This corresponds to the German MAK commission assigning FA into their carcinogen Group 4 with a very similar definition. Therefore derivation of exposure limits should start from the premise that FA is a “threshold” point-of-contact carcinogen. After oral exposure, cytotoxicity of FA led to erosions, ulcerations and hyperplasia in different parts of the stomach (see below) and EFSA (2006) concluded “that such a mechanism may also encompass a thresholded response.“ Similarly, according to Environment Canada, Health Canada (2001) formaldehyde-induced carcinogenicity appears to be a consequence of proliferative regeneration following cytotoxicity and formaldehyde levels sufficiently low to prevent irritation and inflammatory responses therefore present negligible risk (https://www.canada.ca/en/health-canada/services/publications/healthy-living/residential-indoor-air-quality-guideline-formaldehyde.html).

Sustained cell proliferation is the most relevant driver of carcinogenicity, and is governed by the magnitude of localized cytotoxic responses to FA. But a further important factor is the sensitivity of the exact type of epithelial lining exposed to FA. For example, Kerns et al. (1983) observed histopathological alterations of the nose lined with squamous epithelium with a LOAEL of 2 ppm but no tumors originated from this site. According to Morgan et al. (1986) sites of origin of nasal tumors are lined by respiratory epithelium but not by squamous epithelium. Kimbell et al. (1983) developed a three-dimensional model for inhalative airflow of FA in the nasal passages of the rat. For respiratory epithelium there was a good correlation between local airflow impact and sites of tumor origin. But the highest FA impact was seen in the nasal vestibule with a different epithelial lining, a location that was never a site for tumor development. Thus, the carcinogenic response not only depends on the local FA concentration but also to a large extent on the type of epithelium directly exposed to FA.

4. Oral studies in mammals with specific emphasis on local irritation

Histopathological alterations are an important but not the only prerequisite for tumor induction and the sensitivity of the gastrointestinal (GI) epithelium has to be taken into due consideration. As such, since the GI tract epithelium is exposed via the oral route after dietary exposure, derivation of this SML concentrates on studies with oral application.

Local tissue irritation progressing to cytotoxicity at higher doses is the predominant feature of FA toxicity. Studies with oral exposure, either via drinking water or feed, that include reliable dose-response data related to local tissue irritation are summarized in Table 1.

The most reliable study is that of Til et al. (1989) who exposed Wistar rats (70 animals/sex/dose) to FA in drinking water in a combined chronic/carcinogenicity study over 24 months. This is considered the key study by several expert committees (CICAD, 2002; WHO, 2005; BfR, 2006). Target dose levels were 5, 25 and 125 mg/kg bw/d and the drinking water concentrations were adjusted by body weight and water consumption to attain these doses. The doses actually achieved were 1.2, 15 and 82 mg/kg bw/d (males) and 1.8, 21 and 109 mg/kg bw/d (females) calculated by body weight and water consumption. On average, the FA concentrations were 20, 260 and 1900 mg/l drinking water. Hematology, clinical chemistry and urine analyses were carried out at different time intervals. Subgroups of 10 animals/sex/dose were sacrificed at week 53 and 79 for interim organ weight determinations and histopathology.

In the high dose animals body weights were significantly decreased and there was a remarkable reduction of liquid intake (−40%). A reduced intake of food was statistically significant over the whole exposure period in males, but attained significance in females only sporadically. The most important histopathological findings were observed in high dose males and females in the forestomach (focal papillary epithelial hyperplasia, hyperkeratosis and ulceration) and the glandular stomach (chronic atrophic gastritis, focal ulceration, and glandular hyperplasia) that were first observed at the week-53 sacrifice. The limiting ridge was thickened in most high-dose rats. Treatment related tumors did not occur. Thus, toxicity of oral exposure to FA was governed by local irritation of the stomach with a NOAEL of 260 mg/l water corresponding to a dose of 15 mg/kg bw/d (males) and 21 mg/kg bw/d (females).

Dose selection for the chronic study was based on the 4-week drinking water study of Til et al. (1988) again with Wistar rats at 5, 25 and 125 mg/kg bw/d. The drinking water concentrations were not given as they had been adjusted to the body weight increase during the exposure period to achieve a predetermined dosage in mg/kg bw/d. Using the conversion factors of EFSA (2012) for subacute studies, the drinking water concentrations are estimated to average to about 42, 210 and 1040 mg/l. Parameters evaluated included hematology, clinical chemistry, urine analysis, organ weights and histopathology of several organs. Systemic effects were likely related to palatability or

Table 1
Repeated dose oral studies with specific emphasis on local irritation.

Reference	Duration	Route	Species (strain)	No of animals/ group	Dose[a]		Local effects	Local NOAEL	
					mg/l w or mg/kg feed	mg/kg bw/d		mg/l w or mg/kg feed	mg/kg bw/d
Studies in mammals									
Til (1989) [c]	2 y	w	Rat (Wistar)	70 m/70 f[b]	20, 260, 1900 mg/l[c]	**1.2, 15, 82 (m) 1.8, 21, 109 (f)**	Top dose: forestomach: hyperkeratosis, epithelial hyperplasia, focal ulceration. Glandular stomach: ulceration, hyperplasia	260	**15 mg/kg (m); 21 mg/kg (f)**
Til (1988) [c]	4 wk	w	Rat (Wistar)	10 m/10 f	42, 210, 1040[d]	**5, 25, 125**	Top dose: hyperkeratosis forestomach; focal gastritis glandular stomach	210[d]	**25**
Johannsen (1986) [c]	91 d	w	Rat (SD)	15 m/15 f	555, 1110,1670[d]	**50, 100, 150**	Gastrointestinal mucosa appeared normal, but no information on histopathology	1670[d,h]	**150**[h]
Takahashi (1986)	32 wk	w	Rat (Wistar)	10 m	**1500 or 5000** [g]		Erosions and ulcers at the limiting ridge; papillomas in 8/10 rats	Not determined	
Tobe et al., (1989)	2 y	w	Rat (Wistar)	20 m/20 f[b]	**200, 1000, 5000**	10, 50, 300	Top dose: forestomach: hyperkeratosis, epithelial hyperplasia, erosion, ulceration. Glandular stomach: erosion, ulceration, hyperplasia. In single animals at 1000 mg/l forestomach hyperkeratosis	**200**	10
Johannsen (1986) [c]	91 d	feed	Dog (Beagle)	4 m/4 f	varied[c]	**50, 75, 100**	Gastrointestinal mucosa appeared normal, but no information on histopathology		**100** [h]
Studies in birds									
Babar (2001)	7 wk	feed	Broiler chicken	20 m	**925, 1850, 3700, 7400**	135, 252, 603, 1370[e]	Necrosis, ulcers in crop and proventriculus at the highest dose; Petechial hemorrhages in small intestine at ≥1850 mg/kg feed	**925**	
Anwar (2001)	8 wk	feed	Male quail	15 m	**925, 1850, 3700, 7400**	221, 306, 629, 1210[e]	No gross lesions in glandular or muscular stomach or intestines. No histo.	**7400; no histo**	
Khan (2005)	8 wk	feed	Female quail	15 f	**925, 1850, 3700, 7400**	182, 352, 691, 1324[e]	Stomach: ulceration at the highest dose and macroscopic alterations at ≥3700 mg/kg feed	**1850**	
Khan (2003)	8 wk	feed	Cockerel	15 m	**925, 1850, 3700**	59, 114, 220,[e]	Erosions of crop mucosa at the highest dose	**1850**	
Khan (2003)	8 wk	crop	Cockerel	15 m	**11,100**	56, 102, 203, 251[f]	Histopathological lesions of the crop starting at 102 mg/kg bw		56

In bold letters exposure metrics given by the authors, in normal letters metrics calculated here for comparison purpose.
m: male; f: female; w: water; crop: direct application into crop; histo: histopatology; d: days; wk: weeks; y: years.

[a] dose levels for drinking water studies in mg/l water; for application via feed in mg/kg feed.
[b] Interim sacrifice after 12 and 18 months, each 10 m/10 f (Til, 1989); each 6 m/6f (Tobe et al., 1989).
[c] Concentrations in drinking water or feed were adjusted to achieve a predetermined dose based on mg/kg bw.
[d] Estimated using the factors of EFSA (2012) for conversion of mg/kg bw/d to drinking water concentration for subacute or subchronic studies.
[e] Estimated from body weight and feed consumption at study week 4.
[f] Estimated from body weight at week 4.
[g] dose unclear, since it could not be determined if the concentration listed was for formalin or formaldehyde.
[h] Unclear whether stomach was subjected to histopathology, cf. text.

secondary to GI irritation with decreased food and water intake at 125 mg/kg bw/d while weight gain was not affected. The most important effects were noted in the stomach at 125 mg/kg bw/d: hyperkeratosis of the forestomach, focal gastritis in the glandular stomach and thickening of the limiting ridge. The NOAEL was 25 mg/kg bw/d, corresponding to 210 ppm in drinking water, in concordance to the corresponding concentrations in the 2-year study.

A similar result was reported by Johannsen et al. (1986) with Sprague Dawley rats exposed over 91 days via drinking water at 50, 100 and 150 mg/kg bw/d. Drinking water concentrations were not reported and modified to achieve the preselected doses based on body weight. Again, by the conversion factors of EFSA (2012) for subchronic studies, the drinking water concentrations are estimated to average 555, 1110 and 1670 mg/l. The set of parameters evaluated was similar to that of Til et al. (1988) and over 20 tissues were studied by histopathology (not further specified). Body weight was significantly reduced at 150 (both sexes) and 100 mg/kg bw/d (males) leading to a systemic NOAEL of 555 mg/l. It was stated for all dose levels that "the gastrointestinal mucosa … appeared normal", but it is unclear whether the stomach was subjected to histopathology. Therefore, a local NOAEL would have been 1670 mg/l but this estimate is surrounded by uncertainty.

Takahashi et al. (1986) studied the formation of gastric tumors in an initiation/promotion experiment with male Wistar rats. Initiation consisted of the combined treatment with N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) in drinking water (100 mg/l) and NaCl (10%) in the diet over 8 weeks. Subsequently, FA was given in the drinking water over 32 weeks either with or without preceding initiation. The dose is not clear: it was specified as "0.5% formalin (formaldehyde)" and therefore the FA concentrations might either have been 5000 or 1500 mg/l (assuming formalin with 30% FA). Histopathology concentrated on the stomach.

FA treatment led to significantly increased neoplastic effects in different stomach regions. The incidences after initiation and subsequent treatment with vs. without FA, respectively, were (number of tumor bearing rats/total number of rats):

- Forestomach: papillomas 15/17 vs. 0/30
- Fundus: adematous hyperplasias 15/17 vs. 0/30
- Pylorus: adenocarcinomas 4/17 vs. 1/30

It was concluded that after the combined initiation with MNNG and NaCl, FA acts as a promotor, especially in the pyloric region. This finding supports the concept that FA induced cytotoxicity enhanced the growth of the initiated cell population.

Without initiation, FA at 1500 or 5000 mg/l (use of formalin or formaldehyde unclear) over 32 weeks led to decreased body weight, but far less than in the initiation/promotion study. Papillomas were observed in the forestomach (8/10 vs. 0/10 in untreated controls). The incidence of papillomas was very similar to that of the initiation/promotion part, 80% vs. 88.2%, respectively. In the limiting ridge between fundus and forestomach both erosions and ulcers were found with FA alone as well as after initiation and promotion.

Til et al. (1989) discuss the discrepancy of local effects observed in their study and that of Takahashi et al. (1986), namely formation of papilloma. They offer two explanations, either the use of Wistar rat strains with different sensitivity or the application of different criteria for the classification as papilloma or papillary epithelial hyperplasia which often are difficult to distinguish. The first assumption was strengthened by the finding that the non-neoplastic mucosal changes described by Takahashi et al. (1986) were more severe than those observed by Til et al. (1989), in spite of the longer duration of the latter. An unequivocal decision about the potential influence of different histopathological criteria would only be possible by a reevaluation of the slides of both studies. Since forestomach tumors are not generally considered relevant for assessing human risk (Proctor et al., 2007), and substantial information exists that demonstrate an Adverse Outcome Pathway by cytotoxicity with regenerative cell proliferation AOP to be likely applicable for formaldehyde (Proctor, 2018), the differences in results for forestomach papillomas do not result in additional uncertainties in this assessment.

Tobe et al. (1989) reported the results of a chronic oral toxicity study in Wistar rats (20 rats/sex/dose) at constant drinking water concentrations of 200, 1000 and 5000 mg/l of formaldehyde, estimated to correspond to 10, 50 and 300 mg/kg bw/d. It is noted that the mid and high dose levels would come close to the high dose of Takahashi et al. (1986) irrespectively of the uncertainty of the latter, i.e. 1500 or 5000 mg/l. Interim sacrifices were carried out at 12 and 18 months (6 animals/sex/dose). Parameters investigated corresponded to those required for chronic toxicity studies. The top dose led to a poor condition of the animals and mortality was increased starting already after day 9 of exposure, reached 50% at 12 months and 100% after 24 months. Histopathological lesion were observed in top dose males and females in the forestomach (squamous cell hyperplasia, hyperkeratosis, basal cell hyperplasia, erosion/ulcer and submucosal cell infiltration) and in the glandular stomach (glandular hyperplasia, erosion/ulcer and submucosal cell infiltration). In the 1000 mg/l group forestomach hyperkeratosis was described in 1/6 (males) and 1/8 (females) sacrificed at 18 and 24 months. The authors concluded that the NOAEL was 200 mg/l (10 mg/kg bw/d) and that the forestomach was more sensitive than the glandular stomach. Similarly to Til et al. (1989), no indications of carcinogenicity were observed in this study.

Soffritti et al. (1989, 2002) exposed Sprague-Dawley rats to FA (containing 0.3% methanol) in drinking water at dose levels of 10, 50, 100, 500, 1000 and 1500 mg/l. Animals were treated over 104 weeks and then kept until spontaneous death (up to 168 weeks). Besides significantly increased tumor incidences of the GI tract, also a significant increase in hematopoietic neoplasias (including leukemia and lymphoma, not otherwise specified) were reported. Important limitations of the study were noted by BfR (2006), EFSA (2006) and IARC (2012), especially the different incidences of neoplasias reported in both publications (Soffritti et al., 1989 vs. 2002) although both evaluations were based on the same animal experiment. The tumor incidences of neoplasias of the hematopoietic system and of the GI tract were nearly twice in the later publication as compared to the original one. Since no explanations (such as e.g. extended evaluations by higher numbers of tissue sections examined) were given, the extraordinary and unexplained increase in the reported tumor incidence raises severe concern regarding the validity of both publications. A drinking water study only reported in Soffritti et al. (1989) with exposure starting in utero and subsequently lasting over 2 years will not be discussed further, because only one dose level was used (2500 ppm), the results were mentioned as preliminary and a reevaluation has not been reported.

Johannsen et al. (1986) administered FA to Beagle dogs (4 animals/sex/dose) over 90 d at dose levels of 50, 75 and 100 mg/kg bw/d (It is noted that in Table 2 of the study dose levels of FA are reported as 50, 100 and 150 mg/kg bw/d.). FA was added to the feed to achieve the predetermined dose levels based on body weight. At dose levels exceeding 100 mg/kg bw/d dogs either did not consume the daily feed rations or regurgitated the ingested feed. Hematology, clinical chemistry, urine analysis and organ weights were studied and histopathology of over 30 tissues was carried out. Body weight gain was impaired at the highest dose. At all dose levels feed consumption (at the lowest dose only for females) and feed efficiency were decreased. The GI mucosa "appeared normal" but it is unclear whether the stomach was included in the histopathological assessment. Based on weight gain, the NOAEL was 50 mg/kg/day, but a local cytotoxicity NOAEL cannot be defined with certainty.

In summary, due to the important uncertainties, the results of Soffritti et al. (1989, 2002) are not taken into consideration. An important uncertainty exists for local irritation in the investigation of Johannsen et al. (1986) with rats and dogs as it is unclear whether the GI tract was subjected to histopathology. The discrepancies regarding

formation of papilloma between the studies of Takahashi et al. (1986) on the one hand and Tobe et al. (1989) and Til et al. (1989) on the other hand remain unexplained. Apart from this, the repeated oral dose studies showed a very consistent pattern. The target organ was the forestomach and to a lesser degree the glandular stomach. At high exposures unspecific signs of toxicity occurred in rats and dogs, such as decreased body weight and feed and water intake, likely due to GI tract irritation and/or cytotoxicity. The local NOAELs are very close to each other in the two chronic studies. After 2 years of exposure the NOAEL for irritation in the stomach was 15 mg/kg bw/d for males and 21 mg/kg bw/d for females, corresponding to 260 mg/l drinking water on average (Til, 1989).

5. Studies with oral exposure in birds, specific emphasis on local irritation (Table 1)

Data on local irritation after oral exposure in birds are listed in Table 1 along with repeated dose oral studies in mammals. A series of studies on different endpoints using similar dose regimes were carried out in broiler chicks (Babar et al., 2001), in male Japanese quails (Anwar et al., 2001), female Japanese quails (Khan et al., 2005) and White Leghorn cockerels (Khan et al., 2003, 2006). In general, 2.5, 5, 10 or 20 ml formalin (37% FA) were added to 1 kg feed corresponding to 925, 1850, 3700 and 7400 mg/kg feed. Animals were exposed over 8 weeks (or 7 weeks; Babar et al., 2001) and body weights and feed consumption were recorded weekly. In addition, in some studies 3% formalin (corresponding to 11,100 mg FA/l) was directly administered into the crop (Khan et al., 2003, 2006). To enable a dose comparison with other oral studies, the exposures and body weights at week 4 are used here to calculate the dose in mg/kg bw/d. It is noted that in these studies the FA content in feed was not analytically verified but the FA/feed mix was prepared daily in the morning and stored in polypropylene bags (Anwar et al., 2001; Khan et al., 2003), a procedure assumed for all studies of this group. This approach may have limited evaporation or binding of FA to feed constituents to a certain (but unknown) extent.

When Babar et al. (2001) exposed broiler chicks (20/dose group, age not reported) to formalin in feed the highest dose led to significantly reduced feed intake at weeks 3–8 and starting at week 4 also in the other groups apart from that with the lowest exposure (925 mg/kg bw/d). Depressed body weight followed a similar pattern. The highest dose led to necrosis and ulcerations in the crop and proventriculus with a NOAEL of 3700 mg/kg feed. In addition, the small intestine exhibited petechial hemorrhages in the 3 highest dose groups. Based on the findings in the small intestine the NOAEL for local effects was 925 mg/kg feed. Anwar et al. (2001) fed male Japanese quails (15/dose group) at the same dose levels starting at the age of 35 days. No gross lesions were observed in the GI tract, but histopathology was not performed. In a similar study with female quails (15/dose group) starting at the age of 3 days (Khan et al., 2005) ulcerative lesions were observed at the highest dose level (7400 mg/kg feed) and the mucosa of glandular and muscular stomach was harder at ≥3700 mg/kg feed. Based on the latter finding the NOAEL for GI irritation was 1850 mg/kg feed.

Khan et al. (2003) treated White Leghorn cockerels (age 10 weeks, 15/group) with formalin in feed at 925, 1850 and 3700 mg/kg feed or directly administered a 3% solution of formalin into the crop (5, 10, 15 and 20 ml formalin/bird). In the feeding study the highest dose led to erosions of the crop mucosa with a local NOAEL of 1850 mg/kg feed. It is noted that after direct application into the crop always the same FA concentrations were used (11,100 mg/l) and for this route the severity of the local effects depended on the amount of FA given in mg/kg bw/d with erosions on the surface after 10 ml/bird and ulcerations with necrotic tissue at the 2 highest doses. The NOAEL for histopathological lesions of the crop was 56 mg/kg bw/d. It is noted that at the NOAEL the concentration of FA in the crop was the same as in the higher dose groups. But the lower absolute amount of FA may have led to absorption before development of irritative lesions became apparent. In any case, although the point-of-contact effects noted in the crop cannot be directly extrapolated to mammals, they can provide useful comparative information on point-of-contact tissue irritation.

In summary, in birds with 7 or 8 weeks of exposure there is good evidence that the GI mucosa is less sensitive than that of mammals, the NOAELs being ≥1000 mg/kg feed. It cannot be excluded that the actual concentrations were less, potentially due to evaporation from feed or binding of FA to feed constituents. The information after direct application into the crop should not be used for derivation of safe exposure limits, because an aqueous FA solution was applied at only one very high concentration of 11,100 mg/l. Although the point-of-contact irritation data provided by studies in birds is consistent with that from mammalian studies, due to differences of the alimentary tract between birds and mammals, derivation of safe exposure levels based on local irritation is much better characterized with available mammalian studies.

6. Studies with oral exposure, potential systemic effects

The assessment of potential systemic effects, like fertility, developmental toxicity, immunotoxicity or neurotoxicity, will often depend on indirect evidence or on data from non-guideline studies. Indications for systemic toxicity after oral exposure are listed in Tables 2–5. As derivation of safe exposure levels may either be based on the body burden in mg/kg bw/d or on the exposure concentration in feed or water, in Tables 2–5 both dose metrics are given. For oral exposure FA may either be given by feed or drinking water or by gavage. By gavage relatively high peak blood levels may be attained shortly after dosing in contrast to the more protracted blood concentrations after administration in feed or water that better resemble the kinetics expected for exposure of humans via food. Therefore, extrapolation of results obtained after gavage to humans is surrounded by some uncertainty that cannot be quantitated. Aqueous formalin solutions contain methanol as stabilizer generally at concentrations of 10–15%. Methanol will be metabolized to a large extent via FA and FA derived from methanol will add to its internal dose. Therefore, as an approximation to account for the amount FA derived from methanol the internal dose levels will be increased by 10% to obtain the total dose. 10% is at the lower end of the methanol content in formalin and this takes into account that part of methanol may be excreted without metabolization. This does not apply if the administered FA was prepared from paraformaldehyde. In several studies the source of FA is not stated and, thus, a correction for methanol is not done.

7. Reproductive and developmental toxicity

Duong et al. (2011) published a systematic review on reproductive and developmental toxicity of FA based on data from human populations and *in vivo* animal studies. According to the authors a meta-analysis revealed an increased risk of spontaneous abortion and of all adverse pregnancy outcomes combined in FA exposed women, but differential recall, selection bias or confounding could not be ruled out. Animal studies including all routes of exposure suggested to the authors a positive association between exposure and reproductive toxicity, especially in males. However, recent studies (Swenberg et al., 2013; Yu et al., 2015) show that inhaled formaldehyde is not systemically distributed, indicating any association identified by Duong et al. (2011) is not a causal relationship. A large number of animal studies referenced were also inhalation studies or used FA injection (intraperitoneal, intravenous, intramuscular) that are inappropriate for derivation of a SML. Several studies in mice were said to have used intragastric injection, but actually the studies referenced did not use the oral route: As judged by reviewing the English abstracts, Wang et al. (2002) and Xie et al. (2003) used the intraperitoneal route of exposure, Wang et al.

Table 2
Studies/parameters related to developmental toxicity and fertility after oral exposure.

Author (source of FA)[b]	Species (age at start of study)[i]	Duration/ route	Dose[a]		Parameters studied	Effects	NOAEL[a] (correction for methanol)[c]		Comments (indications for other systemic or maternal effects)
			mg/l w or mg/kg feed	mg/kg bw/d			mg/l w or mg/kg feed	mg/kg bw/d	
Developmental toxicity, studies in mammals									
Marks (1980) (Fo)	Mice	gd 6–15/ gavage		**74, 148, 185**	External, visceral, skeletal malformations	No teratogenic effects at doses leading to maternal toxicity		185 (205)	High maternal lethality at high dose (22/34) and reduced weight gain at low but not at mid dose
Hurni (1973) (Fo)	Dogs	gd 4–56/feed	**125, 375**	3.1, 9.4	Influence on pregnancy, malformations in pups	No adverse effects	375	9.4 (10.3)	Low dosage, probably not reaching MTD; no indication for maternal toxicity
Seidenberg (1986) (?)	Mice	gd 7–13/ gavage		**540**	Maternal mortality, number and early weight of pups, external malformations (screening assay)	No indications for embrio-/fetotoxicity at a dose with clear maternal toxicity		540	High maternal mortality (11/30)
Wickramaratne (1987) (?)	Rat	gd 7–17/ gavage		**100**	Maternal weight gain, litter size, pup mortality and weight gain (screening assay)	Reduced weight gain/litter, no effect on litter size and pup survival		< 100	Defined as a potential fetotoxin, no effect on maternal weight gain
Fertility, studies in mammals									
Cassidy (1983) (Fo)	Rats (m) (10 wk)[i]	1d/gavage		**100, 200**	Weight of testes; sperm head counts and abnormalities	At 200 mg/kg bw increase of sperm head counts and abnormalities; testes weights not affected		100 (110)	No indications for systemic toxicity but gastrointestinal tract not investigated
Ward (1984) (Fo)	Mice (m) (16 wk)[i]	5 d/gavage		**100 or 37**[h]	Sperm morphology in cauda epididymis	No statistically significant increase in abnormal sperm, but in the table significant increase in banana shaped sperm		100 (110) or 37 (41)[h]	The authors caution to draw conclusions from effects at near lethal dose levels
Til (1988)[d] (Pa)	Rat (m + f) (5 wk)[i]	28 d/w		**5, 25, 125**	Organ weight: testis, ovary	No effects related to reproductive toxicity, but local irritation at high dose		125	No indication for reproductive toxicity
Til (1989)[d] (Pa)	Rat (m + f) (5 wk)[i]	2 y/w	20, 260, 1900	**1.2, 15, 82 (m); 1.8, 21, 109 (f)**	At week 53, 79, 105: Organ weight: testis, ovary. Histo: testes, ovaries, epididymis, prostate, uterus.	No effects related to reproductive toxicity, but local irritation at high dose	1900	82 (m), 109 (f)	No indication for reproductive toxicity
Johannsen (1986)[d] (Pa)	Rat (m + f) (not reported)[i]	91 d/feed		**50, 100, 150**	Gonadal weights. Histo of over 20 tissues, but unspecified	No effects related to fertility		150	No indication for effects on fertility
Johannsen (1986) (Pa)	Dog (m + f) (not reported)[i]	91 d/feed		**50, 75, 100**	Gonadal weights. Histo of over 30 tissues, but unspecified	No effects related to fertility		100	No indication for effects on fertility
Vargova (1993) (Fo)	Rats (m) (6 wk)[i]	28 d (5 d/wk)/ gavage		**20, 40, 80**	Weight of testes, prostate	No effects related to reproductive toxicity, but reduced body weight ta 80 mg/kg		80 (88)	No indication for reproductive toxicity
Fertility, studies in birds									

(*continued on next page*)

Table 2 (*continued*)

Author (source of FA)[b]	Species (age at start of study)[i]	Duration/ route	Dose[a]		Parameters studied	Effects	NOAEL[a] (correction for methanol)[c]		Comments (indications for other systemic or maternal effects)
			mg/l w or mg/kg feed	mg/kg bw/d			mg/l w or mg/kg feed	mg/kg bw/d	
Anwar (2001) (Fo)	Quail (m) (35 d)[i]	8 wk/feed	**925, 1850, 3700, 7400**	221, 306, 629, 1210[e]	Weights and histo of testes	Decreased testes weights. Decreased diameters of seminiferous tubules, vacuolization of germinal epithelium. Reduced body weight at the high dose.	For testes weights and histo: 925 (1020); minor histo effects with unclear relevance at 925 (1029)	For testes weights and histo: 221 (243); minor histo effects: < 221 (< 243)	Histopathological processing of testes not optimal
Khan (2005) (Fo)	Quail (f) (3 d)[i]	8 wk/feed	**925, 1850, 3700, 7400**	182, 352, 691, 1324[e]	Egg production and weight; examination of oviduct	Reduced egg production and weight at 7400 mg/kg feed; alterations of oviduct at ≥ 1850 mg/kg feed. At these doses reduced body weight and feed uptake starting at week 3	925 (1020)	182 (200)	Effects on female reproductive parameters associated with decreased body weight and feed uptake; possibly secondary effect
Khan (2003) (Fo)	Cockerel (m) (10 wk)[i]	8 wk/feed	**925, 1850, 3700**	59, 114, 220[e]	Weights and histo of testes	No effects on testes weights. Decreased diameters of seminiferous tubules in all dose groups	Testes: histo: < 925 (< 1020)	Testes: histo: < 59 (< 65)	Histopathological processing of testes not optimal
Khan (2003) (Fo)	Cockerel (m) (10 wk)[i]	8 wk/crop	**11.100**	56, 102, 203, 251[f]	Weights and histo of testes	Reduced testicular weights starting at 203 mg/kg bw and decreased diameters of seminiferous tubules in all dose groups		Testes histo: < 56 (< 62) Testes weight: ↓ 102 (112)	Histopathological processing of testes not optimal
Khan (2006) (Fo)	Cockerel (m) (10 wk)[i]	8 wk/feed	**925, 1850, 3700**	59, 114, 220[e]	Testosterone in blood	Statistically significant decrease at ≥1850, numerical decrease at 925 mg/kg feed	< 925 (< 1020)	< 59 (< 65)	
Khan (2006) (Fo)	Cockerel (m) (10 wk)[i]	8 wk/crop	**11.100**	56, 102, 203, 251[f]	Testosterone in blood	Statistically significant decrease at ≥102, numerical decrease at 56 mg/kg bw		< 56 (< 62)	

In bold letters exposure metrics given by the authors, in normal letters metrics calculated here for comparison purpose.
gd: gestation day; m: male; f: female; w: drinking water; d: day; wk: week; y: year; crop: direct application into crop; histo: histopathology.

[a] dose levels for drinking water studies in mg/l water; for application via feed mg/kg feed.
[b] Test material: Fo = formalin solution; Pa = Paraformaldehyde; (?) = Unknown.
[c] In brackets correction for methanol content by ~10% if formalin was used.
[d] drinking water concentrations were adjusted to achieve a predetermined dose based on mg/kg bw.
[e] Estimated from body weight and feed consumption at study week 4.
[f] Estimated from body weight at week 4.
[h] Contradiction in dose levels reported: unclear whether dose relates to formalin with 37% FA or FA itself (see text).
[i] Age at start of treatment in brackets.

Table 3
Studies/parameters related to immunotoxicity after oral exposure.

Author (source of FA)[b]	Species	Duration/ route	Dose[a]		Parameters studied	Effects	NOAEL[c]		Comments
			mg/l w or mg/kg feed	mg/kg bw/ d			mg/l w or mg/kg feed	mg/kg bw/ d	
Studies in mammals									
Vargova (1983) (Fo)	Rat (m)	28 d (5 d/ wk)/gavage		**20, 40, 80**	Spleen, thymus, lymph nodes: weight, cellularity, immunohistology. Determination of IgG, IgA, IgM. Hemagglutinin and antibody response to sheep erythrocytes. Microbiocidal and phagocytic activity.	All dose levels: increased lymph node weights without effects on cellularity. Reduction of antibody response in hemagglutinin assay without reduction of IgM producing cells in spleen.		< 20 (< 22)	According to authors: possible immune-suppression but further studies necessary.
Til (1988) [d] (Pa)	Rat (m + f)	28 d/w		**5, 25, 125**	Hematology: leukocyte counts. Organ weights: thymus	No effects related to potential immunological parameters		125	No indication for effects on immune system
Til et al., 1989 [d] (Pa)	Rat (m + f)	2 y/w	20, 260, 1900	**1.2, 15, 82 (m); 1.8, 21, 109 (f)**	Hematology (week 26 and 103): leukocyte counts. Organ weights: spleen. Histo: spleen, lymph nodes after 53, 79 and 102 weeks.	No effects related to potential immunological parameters		82 (m); 109 (f)	No indication for effects on immune system
Johannsen et al., 1986 [d] (Pa)	Rat (m + f)	91 d/feed		**50, 100, 150**	Hematology: total and differential leukocyte counts. Histo of over 20 tissues, but unspecified	No effects related to potential immunological parameters		150	No indication for effects on immune system
Johannsen et al., 1986 (Pa)	Dog (m + f)	91 d/feed		**50, 75, 100**	Hematology: total and differential leukocyte counts. Organ weights: spleen. Histo of over 30 tissues, but unspecified	No effects related to potential immunological parameters		100	No indication for effects on immune system
Studies in birds									
Babar et al., 2001 (Fo)	Broiler chicks	7 wk/feed	**925, 1850, 3700, 7400**	135, 252, 603, 1370[e]	Weight of spleen and bursa Fabricius	At high dose: decreased absolute and relative spleen weights; lowest absolute weight but highest relative weight for the bursa	3700 (4100)[g]	603 (663)[g]	No details given for organ weights, therefore levels of significance and dose response relationships cannot be assessed
Anwar et al., 2001 (Fo)	Quail (m)	8 wk/feed	**925, 1850, 3700, 7400**	221, 306, 629, 1210[e]	Spleen weights	Relative spleen weight not affected in a dose response relationship.	7400 (8150)	1210 (1330)	
Khan et al., 2005 (Fo)	Quail (f)	8 wk/feed	**925, 1850, 3700, 7400**	182, 352, 691, 1324[e]	Spleen weights, leukocyte counts	Relative spleen weights reduced at all dose levels without a dose response relationship. Leukocytes decreased without reaching statistical significance			NOAEL cannot be defined because a dose response relationship is missing
Khan et al., 2003 (Fo)	Cockerel (m)	8 wk/feed	**925, 1850, 3700**	59, 114, 220[e]	Histo of spleen	No effects	3700 (4100)	220 (242)	
Khan et al., 2003 (Fo)	Cockerel (m)	8 wk/crop	**11.100**	56, 102, 203, 251[f]	Histo of spleen	No effects		251 (276)	
Khan et al., 2006 (Fo)	Cockerel (m)	8 wk/feed	**925, 1850, 3700**	59, 114, 220[e]	Leukocyte counts	At week 8 leucocytes decreased al all dose levels	< 925 (< 1020)	~59 (< 65)	
Khan et al., 2006 (Fo)	Cockerel (m)	8 wk/crop	**11.100**	56, 102, 203, 251[f]	Leukocyte counts	At week 8 leucocytes decreased al all dose levels		~56 (< 62)	

In bold letters exposure metrics given by the authors, in normal letters metrics calculated here for comparison purpose.
m: male; f: female; w: drinking water; d: day; wk: week; y: year; crop: direct application into crop: histo: histopathology.

[a] dose levels for drinking water studies in mg/l water; for application via feed mg/kg feed.
[b] Test material: Fo = formalin solution; Pa = Paraformaldehyde.
[c] In brackets correction for methanol content by ~10% if formalin was used.
[d] drinking water concentrations were adjusted to achieve a predetermined dose based on mg/kg bw.
[e] Estimated from body weight and feed consumption at study week 4.
[f] Estimated from body weight at week 4.
[g] detailed assessment not possible because no details given for organ weights.

Table 4
Studies/parameters related to neurotoxicity after oral exposure.

Author (source of FA)[b]	Species	Duration/route	Dose[a]		Parameters studied	Effects	NOAEL (correction for methanol)[c]		Comments
			mg/l w or mg/kg feed	mg/kg bw/d			mg/l w or mg/kg feed	mg/kg bw/d	
Studies in mammals									
Til et al., 1988 (Pa)[d]	Rat (m + f)	28 d/drinking water		**5, 25, 125**	Organ weight: brain	No effects related to neurotoxicity		125	No indication for neurotoxicity
Til et al., 1989 (Pa)[d]	Rat (m + f)	2 y/drinking water	20, 260, 1900	**1.2, 15, 82 (m); 1.8, 21, 109 (f)**	At week 53, 79, 105: Organ weight: brain. Histopathology: brain, spinal cord, sciatic nerve.	No effects related to neurotoxicity	1900	82 (m); 109 (f)	No indication for neurotoxicity
Johannsen et al., 1986 (Pa)[d]	Rat (m + f)	91 d/feed		**50, 100, 150**	Brain weight. Histopathology of over 20 tissues, but unspecified	No effects related to neurotoxicity		150	No indication for neurotoxicity
Johannsen et al., 1986 (Pa)	Dog (m + f)	91 d/feed		**50, 75, 100**	Brain weight. Histopathology of over 30 tissues, but unspecified	No effects related to neurotoxicity		100	No indication for neurotoxicity
Studies in birds									
Babar et al., 2001 (Fo)	Broiler chicken	7 wk/feed	**925, 1850, 3700, 7400**	135, 252, 603, 1370[d]	Clinical signs and behavioral alterations shortly after feed uptake	Effects observed at ≥3700 mg/kg feed	1850 (2040)	252 (277)	Reduced feed uptake at ≥252 mg/kg bw; body weight at 1370 mg/kg reduced by > 50%
Anwar et al., 2001 (Fo)	Japanese quail (m)	8 wk/feed	**925, 1850, 3700, 7400**	221, 306, 629, 1210[d]	Clinical signs and behavioral alterations	Effects observed at ≥3700 mg/kg feed	1850 (2040)	306 (337)	Reduced feed uptake at all dose levels at week 8
Khan et al., 2005 (Fo)	Japanese quail (f)	8 wk/feed	**925, 1850, 3700, 7400**	182, 352, 691, 1324[d]	Clinical signs and behavioral alterations	Effects observed at ≥3700 mg/kg feed	1850 (2040)	352 (387)	Reduced feed uptake at the 2 highest dose levels
Khan et al., 2003 (Fo)e	Cockerels (m)	8 wk/feed	**925, 1850, 3700**	59, 114, 220[d]	Clinical signs and behavioral alterations	Effects at 3700 mg/kg feed	1850 (2040)	114 (125)	
Khan et al., 2003 (Fo)e	Cockerels (m)	8 wk/crop	**11,100**	56, 102, 203, 251[f]	Clinical signs and behavioral alterations	Effects at ≥102 mg/kg bw/d		56 (62)	Reduced feed uptake at ≥203 mg/kg bw
Khan et al., 2006 (Fo)e	Cockerels (m)	8 wk/feed	**925, 1850, 3700**	59, 114, 220[d]	Specific signs: dullness, depression, staggering, somnolence, anorexia	Effects at 3700 mg/kg feed	1850 (2040)	114 (125)	
Khan et al., 2006 (Fo)e	Cockerels (m)	8 wk/crop	**11,100**	56, 102, 203, 251[f]	Specific signs: dullness, depression, staggering, somnolence, anorexia	Effects at ≥203 mg/kg bw/d		102 (112)	Reduced feed consumption at ≥102 mg/kg bw

In bold letters exposure metrics given by the authors, in normal letters metrics calculated here for comparison purpose.
m: male; f: female; d: day; w: water; wk: week; y: year; crop: direct application into crop.

[a] dose levels for drinking water studies in mg/l water; for application via feed mg/kg feed.
[b] Test material: Fo = formalin solution; Pa = Paraformaldehyde.
[c] In brackets correction for methanol content by ~10% if formalin was used.
[d] drinking water concentrations were adjusted to achieve a predetermined dose based on mg/kg bw.
[e] Estimated from body weight and feed consumption at study week 4.
[f] Estimated from body weight at week 4.

Table 5
Studies/parameters related to other effects after oral exposure.

Author (source of FA)[b]	Species/Sex/ Route/Duration	Dose[a]		Effects noted at the doses given (related to original dose metric)							
		mg/l w or mg/kg feed	mg/ kg bw/d	Body weight	Consumption						
					Feed	Water	Kidney	Liver	Erythron	Other	Parameters investigated
Studies in mammals											
Johannsen et al., 1986 [c] (Pa)	Rat/m, f/w/91 d	555, 1110, 1670	**50, 100, 150**	↓ 150 f; ↓ ≥100 m	=	↓ ≥50 m, f	=	=	=	= hematology	Hematology, clinical chemistry, urine analysis, OW, histo
Johannsen et al., 1986(Pa)	Dog/m, f/feed/ 91 d		**50, 75, 100**	↓ 100 m, f	↓ ≥50 f; ↓ ≥75 m	Ni	=	=	=	= hematology	Parameters similar to rat study
Takahashi et al., 1986 ?	Rat/m/w/32 wk	**1500 or 5000 ?**		↓ 1500 or 5000 ?	Ni	Ni	Ni	Ni	Ni		Only stomach studied in detail
Til et al., 1988 [c] (Pa)	Rat/m, f/w/28 d	42, 210, 1040	**5, 25, 125**	=	↓ 125	↓ 125	↑ ROW 125 f	=	=	↓ plasma protein, albumin 125 m; = hematology	Hematology, clinical chemistry, urine analysis, OW, histo
Til et al., 1989 [c] (Pa)	Rat/m, f/w/2 y	20, 260, 1900	**1.2, 15, 82 (m); 1.8, 21, 109 (f)**	↓ 82 m; 109 f	↓ 82 m	↓ 82 m; 109 f	histo 82 m; 109 f; ↑ ROW 109 f	= histo and weight	=	↓ plasma protein and cholesterol	Parameters studied similar to 28-day study of Til et al. (1988)
Tobe et al., 1989 (Pa)	Rat/m, f/w/2 y	**200, 1000, 5000**	10, 50, 300	↓ 5000. All rats died at 5000 starting already at d 9	↓ 5000	↓ 5000	= OW, histo; ↑ urea 5000 m, f	= OW, histo; ↓ GOT and GPT 5000 m	↓ (no details given, but not dose related)	↓ plasma protein and cholesterol 5000 m, f; ↓ Plasma protein 1000 m	Hematology, clinical chemistry, OW, histo
Vargova et al., 1993 (Fo)	Rat/m/gavage/ 28 d (5 d/wk)		**20, 40, 80**	↓ 80	Ni	Ni	= histo and OW	= histo and OW	↑ 80	= plasma proteins; ↑ OW lymph nodes ≥40	Histo and weight of several organs, hematology, clinical chemistry
Soffritti et al., 2002 (Fo with 0.3% methanol)	Rat/m, f/w/2 y	**10, 50, 100, 500, 1000, 1500**		=	=	↓ ≥500 f; 1500 m	Ni	Ni	Ni		Only tumor incidences reported
Studies in birds											
Babar et al., 2001 (Fo)	Chicken/sex not specified/feed/ 7 wk	**925, 1850, 3700, 7400**	135, 252, 603, 1370[d]	↓ ≥1850	↓ ≥1850	Ni	= histo	= histo; ↓ ROW ≥1850	Ni		
Anwar et al., 2001 (Fo).	Quail/m/feed/ 8 wk	**925, 1850, 3700, 7400**	221, 306, 629, 1210[d]	↓ ≥3700	↓ 7400	Ni	↓ ROW ≥3700	↓ ROW 7400; ↑ ROW 3700	Ni		
Khan et al., 2003 (Fo)	Cockerel/m/ feed/8 wk	**925, 1850, 3700**	59, 114, 220[d]	=	=	Ni	= histo	= histo	Ni		
Khan et al., 2003 (Fo)	Cockerel/m/ crop/8 wk	**11.100**	56, 102, 203, 251[e]	↓ ≥203	↓ ≥203	Ni	histo ≥56	histo mild effects ≥203	Ni		
Khan et al., 2005 (Fo)	Quail/f/feed/ 8 wk	**925, 1850, 3700, 7400**	182, 352, 691, 1324[d]	↓ ≥3700	↓ ≥3700	Ni	↓ OW 7400	= OW	↓ ≥3700	↑ total serum proteins ≥925; ↓ albumin 7400	
Khan et al., 2006 (Fo)	Cockerel/m/ feed/8 wk	**925, 1850, 3700**	59, 114, 220[d]	Anorexia 3700	↓ 3700 (only at some time points)	Ni	↑ urea, creatinine 3700	↑ ALT, ↓ AST, ↓ AP ≥925	↓ ≥1850	= total serum proteins; = Glucose.	Body weight not measured. Hematology, clinical chemistry

(*continued on next page*)

Table 5 (*continued*)

Author (source of FA)[b]	Species/Sex/ Route/Duration	Dose[a]		Effects noted at the doses given (related to original dose metric)							Parameters investigated
		mg/l w or mg/kg feed	mg/ kg bw/d	Body weight	Consumption		Kidney	Liver	Erythron	Other	
					Feed	Water					
Khan et al., 2006 (Fo)	Cockerel/m/ crop/8 wk	**11.100**	56, 102, 203, 251[e]	Anorexia ≥203	↓ ≥203 (at all time points)	Ni	↑ urea, creatinine ≥102	↑ ALT, ↓ AST, ↓ AP ≥56	↓ ≥102	↓ total serum proteins 251; ↓ glucose 251.	Body weight not measured. Hematology, clinical chemistry

In bold letters exposure metrics given by the authors, in normal letters metrics calculated here for comparison purpose.
↑: increase; ↓: decrease; =: no difference to controls; Ni = no information given; histo: histopathological alterations; (R)OW: (relative) organ weights; w: drinking water; crop: direct application into crop; d: day; wk: week; y: year; m: male; f: female; erythron: red blood cells, hemoglobin, hematocrit.
?: dose unclear, cf. text.

[a] dose levels for drinking water studies in mg/l water; for application via feed mg/kg feed; dose metric selected by the author in bold letters.
[b] Test material: Fo = formalin solution; Pa = Paraformaldehyde.
[c] drinking water concentrations were adjusted to achieve a predetermined dose based on mg/kg bw.
[d] Estimated from body weight and feed consumption at study week 4.
[e] Estimated from body weight at week 4.

(2005) the intraperitoneal and inhalation routes, while Wang et al. (2006) studied effects on testicular cells *in vitro*. For Huang et al. (2002) an English abstract is not available. Therefore these studies do not present any reliable dose-response information for derivation of a SML.

Smith et al. (1983) proposed a selection of 47 positive and negative reference substances for *in vitro* teratogenicity screening and listed four negative studies for FA. Among these negative studies were two (Pushkina et al., 1968; Gofmekler and Bonashevskaya. 1969) that will not be further discussed here. The first one reported changes in ascorbic acid content in dams and fetuses but no malformations after inhalation exposure to 1 and 0.012 mg FA/m^3 (24 h/d). The second publication (referenced by the journal UDC Hyg Sanit (USSR) 34(5): 266–269, 1969) could not be retrieved.

Data on reproductive toxicity with oral dosing in mammals and birds are listed in Table 2. Developmental toxicity was studied by Marks et al. (1980) in mice after administration of FA (containing 12–15% methanol) on days 6–15 of gestation at dose levels of 74, 148 and 185 mg/kg bw/d. The animals were sacrificed on gestation day 18. 185 mg/kg bw/d was lethal to 22/34 pregnant dams and 148 mg/kg bw/d to 1/35 dams. Maternal weight gain was significantly reduced at the low but not at the mid dose. No statistically significant teratogenic effects (external, visceral, skeletal) were detected in the fetuses of the surviving dams with a NOAEL for developmental effects of 185 (corrected for methanol 205) mg/kg bw/d, the highest dose tested.

Hurni and Ohder (1973) investigated potential reproductive toxicity in pregnant Beagle dogs. Dogs were treated daily from day 4–56 after mating with 300 g pellet feed containing 0, 125 or 375 ppm FA corresponding to 0, 3.1 or 9.4 mg/kg bw/d. FA was used as a 40% solution (most probably formalin) that was sprayed on the pellets. It was mentioned that the pellets were promptly consumed within 5–10 min before FA had a chance to volatize. 9, 10 and 9 pregnant females produced 60, 54 and 64 pups at birth with 0, 125 and 375 ppm FA, respectively. Treatment did not affect pregnancy rate, weight gain of the pregnant animals or the size of the 28 litters. Internal or skeletal malformations were not observed in life-born or still-born pups. The NOAEL for maternal and developmental toxicity was 9.4 (corrected for methanol 10.3) mg/kg bw/d, the highest dose tested. The low doses given are noted and clinical symptoms indicative of (slight) toxicity of FA in the sense of a maximally tolerated dose were not reported.

Seidenberg et al. (1986) screened 55 compounds in mice according to the methodology of Chernoff and Kavlock (1982) for teratogenicity. Data from standard teratology tests were available for these substances and the results of a limited method validation indicated this screen was an effective means to identify potential risks to the developing embryo. In general, pregnant mice were treated by oral intubation at dose levels leading to overt toxicity in the dams (significant maternal weight reduction, up to 10% mortality) from gestation day 8–12. Dams were weighted on day 7 and 13 and day 1 postpartum. They were allowed to give birth and pups were examined for abnormalities, counted and weighted on day 1 of birth and day 3. FA (source not mentioned) at a dose of 540 mg/kg bw/d led to high maternal mortality (11/30) but neonate parameters did not indicate a teratogenic potential of FA. When summarizing the results from these 55 chemicals Seidenberg and Becker (1987) concluded that FA was without developmental toxicity. This result was obtained at a maternal dose that would be considered above the Maximally Tolerated Dose.

Wickramaratne (1987) modified the screening assay of Chernoff and Kawlock (1982) to be used in rats. They tested 26 reference chemicals mainly taken from Smith et al. (1983). Dams were dosed from gestation day 7–17. Maternal observations were restricted to body weight measurements on Day 1, 7–17 and 22 of gestation. Offspring observations were limited to litter weights and number of live and dead pups on days 1 and 5 postpartum. The following rules were set to define a negative teratogen (no effect on litter size, survival or postnatal weight gain), a potential teratogen (reduced litter size at birth and reduced survival) or a potential fetotoxin (reduced postnatal weight gain with no reduction

of survival). FA (source not mentioned) was given orally to 9 dams in corn oil at 100 mg/kg bw/d. Maternal weight gain, mean litter size (live + dead), number of live pups (day 1 and 5) and % survival were not affected by treatment but mean % weight gain per litter was reduced in treated animals (48.4 ± 11.5 in controls vs. 27.1 ± 36.1, no indication about statistical significance given). Therefore, FA was assessed as being potentially fetotoxic. It is noteworthy that a dose of 100 mg/kg/day in rats has been shown by other researchers (Table 1) to be highly irritating and cytotoxic; the effects in the GI tract were not evaluated in this study, and irritant effects may have resulted in reduced weight gain.

For studies on effects to the reproductive system the dosing period in relation to attainment of sexual maturity may be relevant. Cassidy et al. (1983) exposed 10 week old male rats (5 males/dose group; young adults) by oral gavage once to FA (100 and 200 mg/kg bw) containing 11–14% methanol. On day 11 the animals were sacrificed, corresponding to the early spermatid stage, testes were weighed and the number of sperm heads and percentage of sperm head abnormalities were determined in testes. At 200 mg/kg bw there was a significant increase in the incidence of abnormal sperm heads but total sperm head counts were also increased. Testes weights were not affected. The NOAEL was 100 (corrected for methanol 110) mg/kg bw/d. The effects on spermatid morphology were suggested to be an indication for mutagenicity, but the simultaneous increases of sperm head counts and abnormalities remain unexplained. It is noteworthy that the GI tract was not evaluated.

Ward et al. (1984) treated 7 male mice at the age of 16 weeks (sexually mature) by gavage with 100 mg/kg bw/d formalin (37% FA, 10% methanol), corresponding to 37 mg/kg bw/d of FA over 5 days. 5 untreated control animals were used. No statistically significant increase in abnormal morphology was detected in sperm in cauda epididymis, while in the table a significant elevation of banana-type sperm is mentioned (this contradiction remains unexplained). According to the authors the biological significance of these changes is unknown and morphological abnormalities were not observed. The same treatment regimen at 250 and 500 mg/kg bw/d was lethal to all animals (10/10). The authors cautioned to make conclusions from this single dose experiment at near lethal dose levels. (It is noted that the dose levels are partially contradictory as in the results section a dose level for FA of 100 mg/kg bw/d was mentioned.) The NOAEL was 37 or 100 (corrected for methanol 41 or 110) mg/kg bw/d. The repeated dose studies of Til et al. (1988, 1989) provide good evidence that FA (derived from paraformaldehyde) after oral exposure does not affect male and female sex organs. In both studies the rats were 5 weeks old, shortly before reaching the age of sexual maturity. After 28 days and 2 years of exposure no effects in histopathology or weights of sex organs were noted (Til et al., 1988, 1989). The highest doses tested were the NOAELs for these endpoints with 125 mg/kg bw/d in the 28-day study and 82 mg/kg bw/d for males and 109 mg/kg bw/d for females in the 2-year study. Similarly, gonadal weights were not affected by treatment in rats or dogs (age of animals not given) over 91 days up to the highest dose level of 150 or 100 mg/kg bw/d being the NOAELs for rats and dogs, respectively, but it is unclear whether these organs have been histopathologically evaluated (Johannsen et al., 1986). In addition, Vargova et al. (1993) noted no effects on weights of testes and prostate at 20, 40 and 80 mg/kg bw/d after treatment of 10 male rats/dose group (6 weeks of age) by gavage with FA (most probably prepared from formalin) over 28 d (5 d/week). Body weight was significantly reduced at the high dose.

Anwar et al. (2001) fed male Japanese quails (15/dose group) at the age of 35 days with a diet containing 925, 1850, 3700 and 7400 mg FA/kg feed (37% FA) over 8 weeks. Japanese quails reach sexual maturity at the age of about 6 weeks. Feed consumption was significantly reduced in the high dose group at all time points (week 1–8) and at week 8 for all dose levels. Body weights were significantly reduced at week 7 and 8 in the high dose group. Relative testes weights were dose dependently decreased and significantly different from controls at ≥1850 mg/kg feed. The mean diameters of seminiferous tubules were significantly smaller for the 2 highest dose groups and vacuolization of the germinal epithelial layer was dose dependently increased, most prominently again in the 2 highest dose groups. According to the interpretation of the authors FA treatment delayed sexual maturity in this species but an influence by reduced feed intake that was statistically significant at the end of exposure cannot be excluded. The NOAEL for testicular weights was 925 (corrected for methanol 1020) mg/kg feed but the isolated minor histopathological effects at this dose were not further discussed by the authors. It is noted that formalin was used for fixation of testes, an approach not optimal as formalin can lead to shrinking and distortion of the tissue. In this study, potential irritation to the GI tract was not histopathologically evaluated.

A similar study with female Japanese quails started at the age of 3 days (Khan et al., 2005). By the end of the 8 weeks treatment period the birds had probably reached sexual maturity. At the highest dose body weight and feed consumption were significantly reduced starting from week 1, and were accompanied with reduced heart (absolute) and kidney (absolute and relative) weights at termination. The absolute oviduct weights were said to be decreased but according to Table 3 of the publication this effect was not statistically significant. At later time points reduced body weights and feed consumption were also observed for other dose groups, for example at 1850 and 3700 mg/kg bw/d already after the 3rd week. Alterations of the GI tract were observed starting at 3700 mg/kg bw/d (cf Table 1). Mean egg production and egg weight were significantly decreased in the high exposure group and area and numbers of mucosal fold were significantly reduced for different parts of the oviduct at ≥1850 mg/kg feed. By microscopic evaluation, the authors concluded that 925 mg/kg feed was the NOAEL for effects on the female reproductive system. As the effects on egg production and weight were accompanied by reduced body weight and weights of some major organs, a secondary effect on female reproductive parameters cannot be excluded.

Khan et al. (2003) also treated White Leghorn cockerels (15/group) starting at the age of 10 weeks over 8 weeks either with formalin at 925, 1850 and 3700 mg FA/kg feed or directly administered a 3% solution of formalin into the crop (5, 10, 15 and 20 ml formalin/bird). These birds reach sexual maturity at about 20 weeks and therefore at the end of treatment they may just have attained sexual maturity. After direct application into the crop body weights were reduced at the 2 highest dose levels as was feed intake. Exposure via feed had no effect on body weight and led to reduced feed intake at 3700 mg/kg feed only at some time intervals. The significant reductions in feed consumption are likely to have arisen from local irritation. It is noted that reduced body weights often occurred only at the next higher dose. In the feeding study, volume and weights (absolute and relative) of testes were not affected, but the diameters of the seminiferous tubules were significantly smaller for all dose groups as compared to controls. The NOAEL for testicular effects was < 925 (corrected for methanol < 1020) mg/kg feed. After direct application into the crop clinical signs occurred in all dose groups apart from the lowest one. Relative and absolute testes weights as well as testes volumes were significantly decreased in the two highest dose groups accompanied with reduced body weights. The diameters of the seminiferous tubules were significantly smaller for all dose groups as compared to controls. The NOAEL for testicular effects was < 56 (corrected for methanol < 62) mg/kg bw/d after application into the crop. Again it is noted that formalin was used for fixation of testes that may lead to artefacts in histopathology. When comparing the nominal exposures to FA after administration via feed or direct injection into the crop, the effects observed by the latter approach were more pronounced. The authors proposed that FA might have evaporated from feed during the interval between diet preparation and its consumption similar to a proposal of Khan et al. (2005). But another possibility might have been that FA partly had reacted irreversibly with feed components and therefore was

not completely bioavailable as free FA. It is not possible to decide between both of these possibilities as no analytical data are presented for the feed-FA mixtures. In conclusion, while the effects on testes weights and volume were accompanied by reduced body weights, the decreased diameter of seminiferous tubules was observed down to the lowest dose levels where body weights were not affected.

Khan et al. (2006) used the same experimental approach as Khan et al. (2003) starting with cockerels at the age of 10 weeks. As data for feed intake and comb area are very similar in both publications, they possibly stem from the same experiment. Although body weight was not reported in 2006, as a plausible approximation the calculations for FA intake (in mg/kg bw/d) are taken from Khan et al. (2003). Severe clinical symptoms were recorded at the highest dose in the feeding part and the 2 highest doses after application into the crop. In the feeding part, feed intake was generally not affected. After application into the crop, feed intake was always reduced in the 2 highest does groups and at some time intervals also at the lower doses. Testosterone levels were dose dependently decreased; the effect was statistically significant at all doses apart from the lowest ones after feed and direct crop application but even the lowest dose groups showed a clear numerical decrease. The NOAELs were < 925 (corrected for methanol < 1020) mg/kg feed for exposure via feed and < 56 (corrected for methanol < 62) mg/kg bw/d after application into the crop.

In summary, malformations were not induced in rats and mice at dose levels reaching maternal toxicity. In a rat screening study some indication for fetotoxicity (i.e., reduced fetal weights) was obtained at doses that likely caused GI irritation. A negative dog study only has limited weight because the dose levels did not reach maternal toxicity. The NOAELs for male and female sex organs were in the range of 100 mg/kg bw/d in mammals when the animals were exposed as (young) adults. The NOAELs were lower when male birds were treated before reaching sexual maturity. If exposure started around the time of sexual maturity a higher NOAEL was found. Female quails before maturity seemed to be less sensitive than male birds. It is difficult to decide whether the histopathological and hormonal changes observed in the reproductive tract of male birds before reaching sexual maturity were secondary to GI irritation, or have to be regarded as a specific effect of FA because they occurred at exposure levels not leading to reduced body weight.

8. Immunotoxicity

For immunotoxicity (Table 3), CICAD (2002) concluded that “based upon the available although limited data, exposure to formaldehyde is unlikely to be associated with suppression of the immune response.” BfR (2006) reached a similar conclusion that “there was no evidence of immunosuppression in mice or of impaired B-cell function in rats.” But these assessments were based to a large extent on studies with inhalation exposure, where the maximum achievable systemic dose is much lower than with oral exposure. Only one study specifically related to immunotoxicity was identified using the oral route. Vargova et al. (1993) administered FA (most probably from formalin) in water by gavage to male Wistar rats (27/dose group, age 6 weeks) over 4 weeks (5 days/week) at dose levels of 20, 40 and 80 mg/kg bw/d. Spleen, thymus, mesenterial and inguinal lymph nodes were weighted, cellularity was determined microscopically and immunoglobulins by immunohistochemistry. Ten further organs were subjected to histopathology. Specific immunological parameters included determination of immunoglobulins (IgG, IgA and IgM) in blood, serum hemagglutinin antibody response and antibody plaque forming cell response to sheep erythrocytes, microbiocidal activity against Candida albicans and phagocytic activity. Body weight was slightly but significantly decreased at the high dose. The percentage of lymphocytes was significantly decreased at the highest dose while the total leukocyte count was not affected. Relative lymph node weights were increased at all dose levels but cellularity was not affected. There was a dose dependent reduction of antibody response (IgG + IgM) in the hemagglutinin assay without reduction in the number of IgM producing cells in the spleen. According to the authors their results indicated to a possible immunosuppressive effect of FA after oral exposure but more detailed analyses would be required to come to a firm conclusion. A potential weakness of the study should be noted namely that histopathology of the stomach was not carried out. Local inflammation might have occurred that could have influenced immunological parameters. Therefore, the study of Vargova et al. (1993) will not be used for derivation of safe exposure levels.

Some parameters that have relevance for immunological effects were investigated in rats in the 4-week and 2-year studies of Til et al. (1988, 1989). Exposure to FA in drinking water over 4 weeks did not affect leukocyte counts in blood or thymus weights up to the highest dose of 125 mg/kg bw/d. Similarly, no effects were noted in the 2-years study at the highest dose level of 1900 mg/l drinking water (corresponding to 82 and 109 mg/kg bw/d for males and females, respectively) for leukocyte counts including the 26 week interim sacrifice or on spleen weight and histopathology of spleen and mesenteric and axillary lymph nodes after 53, 79 and 105 weeks of treatment.

No effects were recorded by Johannsen et al. (1986) in the 3 month feeding studies with rats (up to 150 mg/kg bw/d) and dogs (up to 100 mg/kg bw/d) for total and differential leukocyte counts and spleen weights (only studied in dogs). The range of organs subjected to histopathology was not specified. The predominant effect was decreased body weight at the high dose in both species.

Babar et al. (2001) reported at the highest exposure of 7400 mg/kg feed decreased absolute and relative spleen weights in broiler chicken. At this dose the absolute weight of the bursa of Fabricius was the lowest one of all dose groups while the relative weight was the highest. Although “Table 4” with details on organ weights is mentioned in the publication, it was not published within the paper. Therefore an analysis of a dose-response relationships and statistical significances is not possible. In contrast, Anwar et al. (2001) reported that relative spleen weight was not affected in a dose related manner in male quails up to 7400 mg/kg feed. However, according to Khan et al. (2005) relative spleen weights were reduced at all dose levels (925–7400 mg/kg feed) in juvenile female quails being the lowest at the high dose, but a clear interpretation is not possible as there was no dose-response relationship. Similarly, a dose-response relationship was not apparent for increased serum globulin levels. Leukocyte counts were decreased, especially at 7400 mg/kg feed without reaching statistical significance. Khan et al. (2003) reported in cockerels no effects by histopathological examination of the spleen after administration of FA via feed (up to 3700 mg/kg feed) or directly into the crop (up to 251 mg/kg bw/d) over 8 weeks. Khan et al. (2006) reported reduced leukocyte counts in juvenile cockerels after 8 weeks of exposure via feed or directly into the cop at all dose levels in a dose related manner but the effects, albeit statistically significant, were small. The NOAELs for this parameter were $^{<}$925 mg/kg feed and $^{<}$56 mg/kg bw/d for both of these exposure routes. Unfortunately, local irritation to the GI tract was not investigated in these birds, but histopathological alterations were reported by Babar et al. (2001) after exposure via feed down to the dose of 1850 mg/kg feed.

In summary, standard parameters possibly indicative of effects on the immune system in repeated dose studies in mammals did not reveal any indication for immunotoxicity after up to two years of exposure. On the other hand, Vargova et al. (1993) reported some indication for immunosuppression by some parameters from a large battery of more sophisticated (but not standardized) tests in rats. They did not obtain a clear NOAEL for doses ranging from 20 to 80 mg/kg bw/d and cautioned that more data would be necessary to reach a firm decision. Studies in birds are difficult to interpret because the same group of authors investigated only selected and different parameters in different studies. In a single study there was the statistically significant but small decrease of leukocyte counts in cockerels before sexual maturity down

to the lowest dose at 925 mg/kg feed and 56 mg/kg bw/d after application into the crop. However, no information is available about local irritation in this study. In total, the results do not show any clear pattern of potential immunotoxic effects and changes in immunological parameters may have been secondary to local irritation/inflammation.

9. Neurotoxicity

Findings possibly related to neurotoxicity are listed in Table 4. In the 28-day and 2-year drinking water studies in rats (Til et al., 1988, 1989) no effects on brain weights were recorded after 28 days of exposure up to 125 mg/kg bw/d or after 53, 79 or 105 weeks up to 82 and 109 mg/kg bw/d (for males and females). No histopathological alterations of the brain, spinal cord and the sciatic nerve were observed. Similarly, brain weight was not affected by treatment in rats or dogs over 91 days up to the highest dose level of 150 or 100 mg/kg bw/day, respectively (Johannsen et al., 1986), but it is unclear whether organs possibly related to neurotoxicity were histopathologically evaluated. Clinical symptoms indicative of neurotoxicity were not described in the studies of Til and Johannsen.

In some bird studies clinical signs and behavioral alterations were described that may be discussed as related to neurotoxicity but a clear differentiation is not possible between neurological symptoms (i.e., clinical signs) being indirectly caused by irritation rather than being a direct reflection of neurotoxicity. Babar et al. (2001) described pronounced clinical symptoms in broiler chicken exposed to ≥3700 mg/kg feed shortly after feed intake like somnolence, dullness, sitting with closed eyes and decreased response to disturbance. Significantly reduced body weights and feed intake were also found at the next lower dose. Similar observations were reported by Anwar et al. (2001) in male Japanese quails fed FA at dose levels ≥3700 with a NOAEL of 1850 mg/kg feed. But after 8 weeks of exposure all dose levels (down to 925 mg/kg feed) had led to a significantly reduced feed consumption while a significantly reduced body weight was only found at the highest dose (7400 mg/kg feed). Similar clinical signs and alterations of behavior with anorexia and depression were recorded in female quails at ≥3700 mg/kg feed, a dose level that also led to reduced feed consumption and body weight (Khan et al., 2005). In the study of Khan et al. (2003) no behavioral alterations were recorded at FA dose levels up to 1850 mg/kg feed when the cockerels were exposed via feed, while body weight and feed uptake were not significantly affected up to the highest dose of 3700 mg/kg feed. After crop application behavioral alterations started at 102 mg/kg bw/d with low response towards disturbance and low attraction to feed in the sense of behavioral depression. The depression lasted only over 30–120 min after treatment depending on dose. Significantly reduced body weights and feed uptake started at the next dose of 203 mg/kg bw. Khan et al. (2006) reported dullness, depression, staggering, somnolence and anorexia that may be indirect effects on behavior rather than specific indications for neurotoxicity. These effects were observed at 3700 mg/kg feed and at ≥203 mg/kg bw/d after application into the crop. Feed intake was reduced in these groups, but in the feeding studies only at some time points.

In summary, in mammals no indications for neurotoxic effects were obtained up to an exposure duration of 2 years. The behavioral alterations in birds were observed at dose levels generally associated with GI irritation, reduced body weights and feed uptake and might simply reflect general and non-specific clinical symptoms of toxicity after dosing. They often occurred only shortly after FA exposure and therefore are most probably related to local tissue irritation and do not reflect neurological damage.

10. Other toxicological endpoints

In Table 5 effects related to other endpoints are listed that were not mentioned in the preceding specific Tables 1–4 A consistent pattern emerges for reduced body weights starting at about 50–100 mg/kg bw/d in rats exposed via drinking water or dogs via feed generally accompanied with reduced feed and water uptake at similar dose levels for exposure durations of 3 months and 2 years. Such effects occurred in birds exposed over 7–8 weeks via feed or by direct application into the crop at about 200 mg/kg bw/d (drinking water uptake was not measured in these studies).

In the majority of high dose male and female rats, Til et al. (1989) reported chronic nephropathy, associated with papillary necrosis that tended to occur already at week 53, providing some indication that the kidney is a potential target organ. The kidney findings were accompanied by a higher density of urine, lower urine production, an increase of relative kidney weights (females only) and higher plasma urea at the highest dose level. Relative kidney weights were also increased at 125 mg/kg bw/d in females in the 28-day study of Til et al. (1988). By referring to Elliott (1986), these findings were considered by the authors as secondary to the massive reduction of water intake. But the studies cited by Elliott (1986) do not justify the general conclusion of reduced water intake leading to papillary necrosis (Saker and Kincaid-Smith, 1969; Gunson and Soma, 1983). On the other hand, Vargova et al. (1993) noted no effects on weights and histopathology of kidney and liver up to 80 mg/kg bw/d after treatment of male rats by gavage with FA (most probably prepared from formalin) over 28 d (5 d/week). This dose level led a significant reduction of body weight.

Studies in birds led to conflicting results: while in feeding studies Babar et al. (2001) and Khan et al. (2003) did not describe degeneration or necrotic effects in the kidney at dose levels up to 7400 mg/kg feed, after application into the crop necrotic epithelial cells were observed in the renal tubules down to the lowest dose of 56 mg/kg bw/d (Khan et al., 2003). Relative kidney weights were decreased (in contrast to the findings in the Til studies) in the feeding studies of Anwar et al. (2001) starting at 3700 mg/kg feed and Khan et al. (2005) observed at 7400 mg/kg feed a decreased absolute and relative kidney weight. Finally, the increases of blood urea and creatinine reported by Khan et al. (2006) at 3700 mg/kg feed and direct crop application at ≥102 mg/kg bw/d indicate to the kidney as target organ.

In most studies (when investigated) plasma proteins and cholesterol were decreased at high dose levels (Til et al., 1988, 1989; Tobe et al., 1989; Khan et al., 2006 – after crop application) which may be related to decreased food uptake. In contrast, the increase of total serum protein (Khan et al., 2005) is an isolated finding that remains unexplained. The increase of erythrocytes and hemoglobin reported by Vargova et al. (1993) was not observed in the other studies with mammals. But in birds at high exposures a decrease of the erythron was observed at ≥3700 mg/kg feed (Khan et al., 2005) and by Khan et al. (2006) at ≥1850 mg/kg feed and ≥102 mg/kg bw/d after direct application into the crop.

As reduced body weights at high doses were often accompanied by reduced absolute or increased relative organ weights as findings without specific toxicological relevance, these effects will not be discussed here. Liver enzymes viewed as indicating liver toxicity when increased, were decreased in the study of Tobe et al. (1989). Khan et al. (2006) mentioned in their discussion that the liver enzymes ALT, AST and AP were increased as an indication of liver toxicity. However, this is not supported by the results presented as an increase was only described for ALT while AST and AP were decreased. Thus, an indication for liver toxicity cannot be derived from these findings.

In summary, over all studies and species there was little consistency apart from decreased body weights and feed and water (if measured) intake that most probably were related to irritation of FA and palatability. There were indications that the kidney was affected in rats with histopathological alterations. Increased urea and creatinine noted in some bird studies may also point to the kidney as potential target organ. Unfortunately, water intake was not measured in birds and therefore a decision is not possible whether this effect instead might have be related to dehydration. Effects on the erythron were inconsistent and a

decrease was only noted in birds generally at dose levels at or above 100 mg/kg bw.

11. Genotoxicity

FA is a highly reactive chemical present in every living cell that can readily react with endogenous macromolecules. It leads to genotoxic effects in a variety of *in vitro* systems that are summarized in many reviews, e.g. BfR (2006). It is concluded that the vast majority of results demonstrates that formaldehyde is genotoxic to bacteria as well as to mammalian cells in culture. In mammalian cells the positive genotoxic endpoints include structural chromosomal aberrations, sister-chromatid exchanges (SCE), gene mutations, DNA strand breaks, DNA adducts, DNA protein crosslinks (DPX) and DNA repair. DNA adducts and DNA protein cross links of FA were found in all organs investigated without external exposure due to the ubiquitous occurrence of endogenously produced FA in all living systems. After single or multiple inhalation exposures to stable isotope labelled FA, adducts derived from exogenous FA were only observed at the site of primary contact (nasal epithelium) with a clear non-linear dose-response relationship, but not in blood or organs distal to the portal-of-entry (Lu et al., 2010; Moeller et al., 2011; Swenberg et al., 2013; Yu et al., 2015; Lai et al., 2016; Leng et al., 2019). With regard to the derivation of a SML for food contact materials, effects after inhalation exposure (or after intravenous or intraperitoneal injection) will not be further discussed here as they do not provide genotoxicity data that can directly be extrapolated to the much higher exposures possible via the oral route. For oral exposure only three genotoxicity studies are available.

Migliore et al. (1989) described an increase of micronuclei and nuclear anomalies (karyorrhexis, pyknosis and vacuolated bodies) in cells of the GI epithelium of rats treated once orally with FA (source not mentioned) at 200 mg/kg bw. These effects were noted in the stomach 16, 24 and 30 h after exposure, in the duodenum after 24 and 30 h and in ileum and colon after 30 h. Only cells in the basal layer were analyzed because these are known to be in active proliferation and are thus potentially more sensitive to genotoxic effects. Local irritation was severe with nuclear anomalies, hyperemia and hemorrhage in the stomach reaching a maximum 30 h after treatment. As only one high exposure was investigated a conclusion regarding the interrelation between severe irritation, cytotoxicity and micronuclei formation is not possible.

Morita et al. (1997) exposed mice to FA (from formalin solution) by gavage up to 200 mg/kg bw on one or two consecutive days. Micronuclei were not increased in polychromatic erythrocytes in the bone marrow (2 doses) or in peripheral reticulocytes (1 dose). Under these conditions all animals died after application of 300 mg/kg bw. Even after a double intravenous injection of up to 30 mg/kg bw (about 60% of lethal dose) an induction of micronucleated reticulocytes was not observed.

Ward et al. (1983) briefly reported a cytogenetic study with formalin (FA content not given) and methanol in mice, but to our knowledge this study has never been published in peer reviewed journals. Mice were treated by gavage with a single oral dose of 100 mg/kg bw formalin (possibly corresponding to about 37 mg/kg bw FA). Analysis of metaphase chromosomes in bone marrow showed a large increase in exchanges (Robertsonian translocations) and in aneuploid cells, 40 vs. 10 and 101 vs. 6 (FA treated vs. controls for exchanges and aneuploidy, respectively). The incidence of breaks was not significantly affected. A similar picture emerged after treatment with methanol at 1000 mg/kg bw orally. In comparison to the results of Morita et al. (1997) there appears to be a striking difference: the large increase in aneuploidy in the Ward study should have been reflected as an increase in micronuclei in the Morita study, but this was not the case. This inconsistency between both studies remains unexplained, but the Morita et al. (1997) study was published in a peer-reviewed journal and was a standard micronucleus study, in contrast to the Ward et al. (1983) study that was incompletely documented in a book chapter.

In conclusion, high oral or intravenous exposure to FA at near lethal doses did not lead to systemic genotoxicity (micronuclei) in the bone marrow and peripheral blood of mice. In contrast, at a lower oral dose, an increased incidence of aneuploidy and exchanges was described in the bone marrow of mice. Micronuclei formation was observed in the GI tract after a single oral dose that also resulted in severe GI tract irritation, but this experiment does not allow to analyze the potential interaction between local cytotoxicity and genotoxicity. Overall, the genotoxicity results after oral exposure agree with other studies indicating formaldehyde exerts its cytotoxic effects primarily at the point-of-contact/portal-of-entry and systemic effects are limited by rapid metabolism.

12. Derivation of a SML

12.1. General considerations

A large number of animal, epidemiology and mechanistic studies are available for FA. However, the vast majority are inhalation studies with only limited relevance for derivation of a SML because the body burden of FA in rats after inhalation is only about 4 mg/kg bw/d at exposure concentrations of 15 mg/m^3 based on the inhalation volume of 0.29 m^3/kg bw/6 h of ECHA (2012). It is well known that after inhalation unmetabolized FA will not reach systemic circulation (e.g. Kleinnijenhuis et al., 2013; Moeller et al., 2011). Much higher doses are given in the oral studies reported here. Systemic effects observed in studies with parenteral application are misleading for derivation of an oral exposure limit because the protective barrier at the portal-of-entry with its detoxifying metabolism is bypassed. To conclude, for dietary exposure oral studies are most relevant.

Effects after oral exposure can be dominated by local irritation to the GI tract depending primarily on the concentration of FA in the application media. However, higher doses may lead to systemic exposure and toxicity when detoxification mechanisms are saturated and/or the protective barrier in the GI tract is compromised (e.g., by ulceration). As local irritation and systemic effects would require different UFs, SMLs will be derived separately for local and systemic effects with the lowest SML selected as the protective value for all toxicological endpoints.

When starting from the body burden in mg/kg bw/d the standard approach of EFSA (2012) for assignment of default UFs is to use a factor of 10 each to account for inter-species extrapolation (from rats to humans) and for intra-species variability within the human population (here the consumers). An additional UF may be necessary for exposure duration, e.g. for extrapolation from subchronic to chronic exposure, if long-term studies are not available. The UF for intra-species variability (humans) is divided into two subfactors each of 3.16 for toxicodynamic and toxicokinetic differences. Similarly, the inter-species UF is divided into subfactors of 2.5 for toxicodynamics and of 4 for toxicokinetics, the latter to account for allometric scaling most probably for rat studies although not specifically mentioned. The toxicokinetic/toxicodynamic division of UFs follows the guidance of WHO/IPCS (2005). When starting from the concentration in feed or water, allometric scaling (subfactor of 4 for rats) is not necessary because feed and water consumption directly depend on the basal metabolic rate and therefore are already scaled according to the allometric principle (ECHA, 2012). If possible, this latter approach will be used here because also the SML can be directly expressed in concentrations in food or water.

With regard to toxicokinetic variability, a specific situation exists. Due to its high intrinsic toxicity and its production and occurrence in all living cells, an efficient and highly conserved metabolic detoxification pathway exists in all animal species leading to rapid conversion of FA to formic acid and finally to CO_2. It has been demonstrated that the adult human liver will metabolize 22 mg FA/min, i.e. 31680 mg/d (Owen et al., 1990; Clary and Sullivan, 2001). Cascieri and Clary (1992)

calculated for the total FA production 2450 mg/h (58800 mg/d) based on a half-life of 1.5 min, a mean FA concentration in body water of 2.5 mg/l and a body weight of 70 kg. A similar conclusion was reached by EFSA (2014a) with 0.61 and 0.91 mg/kg bw/min for half-life values of 1.5 and 1 min, corresponding to 2560 and 3820 mg/h for 70 kg body weight (61440–91680 mg/adult/d or 878–1310 mg/kg bw/d). Such high production rates require efficient metabolic degradation pathways to maintain steady state concentrations of about 2.5 mg/l in body water. The metabolism of FA is rapid as indicated by the short biological half-life in blood of 1–1.5 min in different species (McMartin et al., 1979; Rietbrock, 1969). In humans, FA degradation is primarily catalyzed by the glutathione dependent formaldehyde dehydrogenase (FAD, also known as alcohol dehydrogenase 5, ADH5) and S-formyl glutathione hydrolase to formic acid. Formic acid enters the C1-pool or is further oxidized to CO_2 with a longer half-life in plasma. FAD has an important role to protect all tissues in all species against the intrinsic toxicity of endogenous FA and therefore has been assigned a house keeping character by Estonius et al. (1996). FAD has been detected in a broad panel of species and tissues, e.g. in rats and humans (BfR, 2006; Jörnvall et al., 2000) with very low inter-tissue variation for the majority of human tissues (Estonius et al., 1996). In addition, BfR (2006), WHO (Nielsen et al., 2016) and SCOEL (2016) concluded that there is no major biologically relevant polymorphism in humans.

While the first step of FA metabolism (oxidation to formic acid) is very rapid and similar in all species, the second step (oxidation of formic acid to CO_2) is clearly slower with marked species differences. It is well known that methanol toxicity is much more pronounced in humans and monkeys as compared to rats. Methanol is metabolized via FA to formic acid and then to CO_2. The metabolism of formic acid to CO_2 is much slower in primates than in rats. Thereby formic acid may accumulate and lead to acidosis in primates that has not been observed in rats and this is assumed to be the main reason for methanol toxicity in humans (cf. McMartin et al., 1975). It was shown that the species difference to methanol toxicity resides to a large extent in metabolism of formic acid via the folate-dependent C1 pathway being more efficient in rats than in primates. The efficiency of this pathway can be modified by addition or restriction of folate in feed (McMartin et al., 1977) while the catalase peroxidative system does not play a role. The different half-lives of formic acid in different species was quantified by Rietbrock (1969). The half-life was determined to be 55, 77, 67, 32, 22 and 12 min for humans, dogs, cats, rabbits, guinea pigs and rats, respectively, after dosing with sodium formate. Interestingly, the difference in humans and rats amounts to about a factor of 4, corresponding to allometric scaling. Malorny et al. (1965) reported that a single exposure of FA by gavage at 70 mg/kg bw (a total dose in the same order of magnitude as in the drinking water studies) led in dogs to a massive increase in formic acid in blood from about 20 mg/l to a maximum of 129.5 mg/l after 2 h. The blood pH decreased from 7.45 to 7.29 without having returned to baseline after 3 h.

Very long half-lives of formic acid have been reported for humans admitted to hospitals for treatment of methanol poisoning before hemodialysis was initiated. One patient of Osterloh et al. (1986) had a formate half-life of 3.7 h with a serum formate concentration of 550 mg/l and a blood pH of 7.39. Kerns et al. (2002) studied 8 patients with a mean formate concentration of 695 mg/l (range 23–1600) and a mean blood pH of 7.21 (range 7.0–7.38). For 6 patients the elimination half-life of formic acid was 3.4 ± 1.5 h. In the report of Hantson et al. (2005) the half-lives in 9 patients before or in the absence of hemodialysis were 6.04 ± 3.26 h (range 3.25–12.47) with formate concentrations between 14 and 860 mg/l and pH between 6.7 and 7.36. These publications were the basis for the statement of Dhareshwar and Stella (2008) that "formic half-life in plasma is ~1–6 h", a range that was also published by EFSA (2014b). Formate half-lives from cases of methanol poisoning are misleading for the general population. Normal values for formate in humans are much lower than levels after methanol poisoning, i.e. in the range of 10–20 mg/l, similar to those in other species like the dog (Malorny et al., 1965) or the monkey (McMartin et al., 1979). According to Clay et al. (1975) experimental half-lives in monkeys and rats depend on the dose. After application of 50 mg/kg bw sodium formate the half-life in monkeys was 31 min and with 470 mg/kg bw 51 min and in rats with doses up to 100 mg/kg bw 12 min and with 670 mg/kg bw 23 min. Therefore, for the general population the experimental half-life of 55 min reported by Rietbrock (1969) for humans after a dose of 1.17 mMol/kg bw sodium formate (corresponding to 80 mg/kg bw) is much more relevant than the half-lives after methanol poisoning. Similarly, McMartin et al. (1977) reported a half-life in the monkey of 37 min after iv application of sodium formate at 2.5 mMol/kg bw (corresponding to 170 mg/kg bw) similar to that of Rietbrock (1969) for humans.

While metabolism of FA to formic acid is highly conserved in all species, the second step, oxidation of formic acid to CO_2 clearly shows species differences. This uncertainty should be taken into consideration by applying appropriate toxicokinetic subfactors for inter- and intra-species variability.

Due to POM's specific and heterogeneous applications in kitchenware and food processing machines, determining the appropriate food contact area and volume of daily food processing is challenging. As such, rather than develop POM-specific factors, we refer to the EU (2011) standard default assumption that 1 kg food will come into contact with 6 dm^2 surface area, certainly by far exaggerating potential oral exposures for POM. Thereby our derivation of safe exposure levels via exposure to food becomes directly comparable to the SML(T) of EU (2011), 15 mg FA/kg food corresponding to 2.5 mg/dm^2.

12.2. Derivation of safe exposure levels for local effects

Local effects after oral exposure are driven by the concentration of FA in the application media. Irritation to the GI tract, especially to the stomach where the first contact occurs, has been observed in drinking water and oral gavage studies with mammals, as well as in feeding studies in birds. The findings in birds after direct application into the crop are not taken into account because this specific part of the avian alimentary tract does not have a direct counterpart in mammals and because of the very high concentration applied (11,100 mg/l). Modification of dose levels by different application volumes will only lead to covering different surface areas or modifying the contact time with the crop mucosa by the concentrated FA. The local NOAEL in birds exposed via feed is higher as compared to drinking water studies in mammals, but is surrounded by uncertainties because FA was not analyzed in feed and might have evaporated or bound to feed constituents to an unknown extent. Also, FA in water may have access to the GI epithelium more easily than when mixed with food. Taking into account the different dose spacings in studies with mammals, the local NOAELs do not decrease to any appreciable extent with increasing exposure duration and are very close to each other in the two chronic studies. This corresponds to the general observation that irritant effects are concentration and not time dependent apart from short exposure durations. The study of Til et al. (1989) is preferred to that of Tobe et al. (1989) because of the much higher number of animals. Furthermore, the NOAEL of the Til study is only slightly above that of the Tobe study that showed histopathological effects at the next higher dose level only in a few animals. Therefore, the results of the most comprehensive 2-year study of Til et al. (1989) will serve to define the PoD. Although in this study the concentrations in drinking water were varied over the age of the animals to achieve predetermined dose levels in mg/kg bw/d, the mean drinking water concentration over the study duration is considered appropriate to define a NOAEL of 260 mg/l.

Regarding the interspecies default factor of EFSA, local irritation of FA stems from its high chemical reactivity with rapid macromolecular binding within cells, specifically after oral exposure in the gastro-intestinal tract. This mechanism is basically the same for all species and, in the cell, is counteracted by its rapid degradation via FAD. If

derivation of a safe exposure level is based on the experimental drinking water concentration, allometric/toxicokinetic interspecies scaling (UF of 4 for extrapolation from rats to humans) is not necessary as has been shown by ECHA (2012). Interspecies toxicodynamics will depend on intracellular concentrations of formaldehyde and its macromolecular binding. FA will be rapidly metabolized to formic acid which may also play a role for intracellular toxicity before it is the oxidized to CO_2 by normal C1 metabolism being very similar over all mammals. These considerations may justify a reduction of this subfactor, but as quantitative data especially for the second step are not available, the default factor for interspecies toxicodynamics of 2.5 will be retained. This also takes into account possible differences in the protective mucus barrier.

As regards the intraspecies default factor of EFSA, toxicodynamics for local irritation depend primarily only on the high reactivity of FA and should be very similar all over the human population. But as differences in the mucus barrier cannot be excluded and the intracellular effect of formic acid is unknown, the subfactor of 3.16 will not be changed as a conservative approach. On the other hand, because the mechanism of local toxicity is based on the (unspecific) high chemical reactivity of FA and because of the highly conserved and rapid detoxification of FA, the toxicokinetic subfactor can be reduced to 1.

In conclusion, with the PoD of 260 mg/l and the total UF of 8 (i.e., interspecies 2.5 x intraspecies 3.16 = 7.9) the safe exposure level is 32.5 mg/l or 32.5 mg/kg food, corresponding to 0.46 mg/kg bw/d (adult body weight of 70 kg). With the surface area of 6 dm^2 the SML is 5.42 mg FA/dm^2. For exposures apart from water it has to be taken into consideration that a large part of FA entering the food chain will bind to constituents in food, adding to the conservatism of this approach.

12.3. Derivation of safe exposure levels for systemic effects

A different situation exists for systemic effects after oral exposure than for after inhalation exposure. As demonstrated above, the potential systemic body burdens for the oral route are much higher. At such, two unique factors may come into play:

1. Although not very likely, at high local doses FA may not completely be metabolized at the portal-of-entry and may escape into the whole body, but here again it will be rapidly detoxified with a half-life of 1–1.5 min.
2. Formic acid produced as the first step metabolite by FAD will reach systemic circulation and due to its longer half-life may lead to metabolic acidosis.

Especially the second point needs to be taken into consideration. It has been shown by Malorny et al. (1965) that a single dose of 70 mg FA/kg bw leads to acidosis in dogs. This is a dose level reached in many experimental studies reported here, although it should be noted the dogs were exposed to a bolus dose via gavage, the kinetics of which will certainly differ from those after protracted exposures via drinking water, feed or food. Unfortunately, blood pH is rarely determined in standard toxicological tests and the potential consequences of prolonged acidosis are unknown. Therefore, it cannot be excluded that metabolic acidosis after continuous oral FA exposure may entail toxic effects hitherto unknown while the acute effects in primates are well documented from high dose methanol exposure. Uncertainties surrounding inter- and intra-species toxicokinetics and toxicodynamics impacts the selection of appropriate subfactors. Especially, there is no database to estimate differences in toxicodynamics related to acidosis and therefore the same subfactors of 2.5 and 3.16 used for point-of-contact effects are retained for inter- and intra-species variability related to systemic toxicity.

With regard to intra-species toxicokinetics, the detoxification of formate to CO_2 comes into play while the first metabolic step (oxidation of FA to formic acid) is very similar over all mammals and individuals with no indication for polymorphism. In the second step, formate is metabolized by the physiological C1 pathway that is functionally similar in all individuals. This should lead to a reduction of the kinetic subfactor. As shown by McMartin et al. (1977) formic acid is metabolized by a folate dependent metabolism in primates. After treatment of monkeys with a folate deficient diet over 14 weeks, liver folate concentration had decreased dramatically by a factor of about 5. This was accompanied by an increase of the half-life of formate and a decrease of formate metabolism to CO_2, each by a factor of about 2. After a very long pretreatment of primates over 20 weeks with a folate deficient diet, the effects were slightly more pronounced with a further decrease of liver folate and a reduction of formate metabolism by a factor of about 2.4. In contrast, increasing the plasma folate concentration by a factor of 2000, by ip administration of folate, led to an increase in the rate of formate oxidation by about 50%. Therefore, the toxicokinetics of formate are governed by the availability of folate. By extrapolating the data from monkeys after 14 weeks of folate deprivation with massive depletion of its liver concentration to humans, an intraspecies toxicokinetic subfactor of 2 should be sufficiently conservative. As such, a total UF of 15.8 is derived based upon the toxicodynamic UFs of 2.5 and 3.16, and a toxicokinetic sub-factor of 2.

For derivation of safe exposure limits the oral database has been analyzed and two potential targets far off the portal-of-entry have been identified, the kidney and the gonads.

*Kidney as potential target (*cf. Table 5*)*: In the 28-day and 2-year rat study (Til et al., 1988, 1989) indications for kidney toxicity were observed. The authors discussed this effect as probably related to decreased liquid intake and dehydration due to palatability and therefore not being a toxic effect specific for FA. However, the literature they cited does not support their assumption (Elliott, 1986). It cannot be excluded that kidney toxicity was caused by prolonged metabolic acidosis. The significantly increased urea level at the highest dose of 5000 mg/l drinking water after 12 months in the study of Tobe et al. (1989) point to the kidney as target, too. There are also some findings in the 8-week bird studies indicative of effects on the kidney. While in the feeding studies no histopathological lesions were reported, after direct application into the crop Khan et al. (2003) observed necrotic tubular epithelial cells down to the lowest dose group of 0.56 mg/kg bw. In only one bird study serum urea and creatinine were measured showing an increase at high dose levels after application via feed or directly into the crop (Khan et al., 2006). In total, based on these findings derivation of a safe exposure level should take kidney effects into consideration as a conservative approach. If the drinking water NOAEL of 260 mg/l observed by Til et al. (1989) is taken as PoD, the inter-species toxicokinetic subfactor is 1.

Combining the PoD and NOAEL from Til et al. (1989) with the total UF of 15.8, safe exposure levels of 16.5 mg/l or 16.5 mg/kg food are identified, that equate to a dose of 0.24 mg/kg bw/d (adult body weight of 70 kg). Assuming a surface area of 6 dm^2 the SML is 2.74 mg/dm^2 for potential systemic effects [(16.5 mg/kg food x 1 kg food/day) ÷ 6 dm^2].

In the study of Til et al. (1989) the drinking water concentrations were adjusted to achieve a predetermined constant dose related to body weight. Therefore, a safe exposure level may also be derived based on this dose metric. The lowest NOAEL was found in males with a mean of 15 mg/kg bw/d that then has to be scaled from rats to humans by the allometric factor of 4 according to EFSA (2012). The other sub-factors (combined value of 15.8) remain unchanged as for the calculation based on the drinking water NOAEL. With a total UF of 63.2, the safe exposure level is 0.24 mg/kg bw/d and for an adult identical to that derived by the drinking water NOAEL.

*Sex organs as potential target (*cf. Table 2*)*: There are indications that sex organs may be potential targets in juvenile birds, especially the testes. Studies in rats and dogs started with young adult animals and no

indications for adverse effects in these tissues were obtained. In contrast, some studies in birds were carried out before sexual maturity. The 8-week treatment of cockerels started at the age of 10 weeks (Khan et al., 2003, 2006), that of female Japanese quails at 3 days (Khan et al., 2005) and that of male Japanese quails at 35 days (Anwar et al., 2001). Cockerels reach sexual maturity at about 20 weeks and Japanese quail around 6 weeks. Thus, treatment of cockerels and female quails took place before maturity, and in male quails the start was around that time. Without consideration of potential differences between birds and mammals, which are difficult to specify from toxicological experience, there is some evidence that immature animals may be more vulnerable than mature ones. This is supported when comparing the NOAELs for testicular alterations of immature cockerels (Khan et al., 2003, 2006) with those of quails reaching maturity (Anwar et al., 2001). In quails at this age the NOAEL was 925 mg/kg feed. In comparison, by treatment of (clearly) pre-pubescent cockerels the NOAEL for testicular effects was below the lowest dose after feeding (< 925 mg/kg feed) as well as after direct application into the crop (< 56 mg/kg bw) based on histopathological findings (Khan et al., 2003) and decreased testosterone levels (Khan et al., 2006). Female sex organs of very young quails (3 days at start) seem to be less sensitive and the NOAEL was 925 mg/kg feed for alterations of the oviduct (Khan et al., 2005). The NOAELs after direct application into the crop are difficult to interpret because this specific part of the avian alimentary tract does not have a direct counterpart in mammals and because of the very high concentration applied (11,100 mg/l) while dose levels were modified only by different application volumes. Two general uncertainties of the bird studies are mentioned here again: the concentrations of FA in feed were not analytically verified and fixation of testes for histopathology was not optimal. In summary, the PoD will be based on the NOAEL for testicular effects in feeding studies at < 925 mg/kg feed and after correction for the methanol content in formalin at < 1020 mg/kg feed. The same PoD would apply for indications of immunotoxicity (decreased leukocyte counts), albeit limited, in immature cockerels (Khan et al., 2006).

The same UFs for inter- and intra-species variability are used here as for assessment of effects on the kidney and allometric scaling is not necessary when starting from the concentration in feed corrected for the methanol content. An UF for duration of treatment is not necessary because the most sensitive phase in birds before maturity was covered in the experiments and there is no indication from long-term studies that gonads or leukocytes are affected in mammals after maturity. However, an additional UF is necessary for extrapolation from LOAEL to NOAEL. No specific guidance is given by EFSA (2012). Therefore, an UF of 3 is taken as proposed by ECHA (2012) for the majority of cases as the effects still noted at the LOAEL were of minor severity. The total UF is 47.4 leading to a safe exposure level of 21.5 mg/kg food. Under the assumptions above this translates to 0.31 mg/kg bw/d and to a SML of 3.59 mg FA/dm^2. However, it is recognized that bird studies are poor models for humans. But as these provide the only high-dose oral information about potential effects before sexual maturity, they are used to provide a conservative estimate for potential effects related for reproductive toxicity.

In summary, the following safe exposure levels in mg/kg food, in mg/kg bw/d for adults and SMLs in mg/dm^2 are obtained for the different endpoints and all are above the SML(T) of 15 mg/kg food of EU (2011):

- Local irritation: 32.5 mg/kg food; 0.46 mg/kg bw/d; 5.42 mg/dm^2
- Effects on kidney: 16.5 mg/kg food; 0.24 mg/kg bw/d; 2.74 mg/dm^2
- Effects on testes (and leukocytes) in immature animals: 21.5 mg/kg food; 0.31 mg/kg bw/d; 3.59 mg/dm^2

The lowest safe exposure level and SML obtained for effects on kidney in the long-term rat study of Til et al. (1989) will be used for comparisons with potential exposures.

12.4. Comparison of safe exposure levels with internal and external exposure

Based on former considerations of Cascieri and Clary (1992) and Owen et al. (1990) and half-lives of 1 and 1.5 min, EFSA (2014a) estimated the endogenous turnover of FA to be between 0.61 and 0.91 mg/kg bw/min, i.e. 878 and 1310 mg/kg bw/d. The lowest safe exposure level of 0.24 mg/kg bw/d that should be the basis for safety assessment for FA leaching from POM is by a factor of about 3700 and 5500 lower than the endogenous daily production, demonstrating the conservative nature of our approach.

Background levels in food products are very variable and range from 1 mg/kg in milk to > 200 mg/kg in some fish species or in the outer layer of smoked ham. In summary, EFSA (2014a) assumed that oral exposure to FA in humans from dietary sources is unlikely to exceed 100 mg/adult/d corresponding to about 1.4 mg/kg bw for a body weight of 70 kg. Owen et al. (1990) estimated the daily intake to be somewhat lower, about 11 mg/d, corresponding to 0.16 mg/kg bw/d. Tables with FA content in different food items are given for example by Owen et al. (1990) and EFSA (2014a). In addition, consultation of the EFSA Comprehensive European Food Consumption Database to estimate consumer exposure to different food items led EFSA (2014b) to the conclusion that the maximum intake of consumers (high consumers of meat and milk) would be 4.1 mg FA/person/d (0.06 mg/kg bw/d). This estimated background human exposure is over an order of magnitude below the EFSA (2014a) estimate and four fold lower than the lowest safe exposure level of 0.24 mg/kg bw/d derived in this manuscript.

Migration data for formaldehyde in Hostaform® (i.e., stabilized POM from Celanese) from a study that followed standardized procedures (EN 13130-1 and EN 1313023) are available (Knuppe, 2005) that provide reliable data for potential transfer of FA from POM into food (cf. Table 6). Knuppe (2005) demonstrated very low migration into fatty food as extraction with olive oil always was below the detection limit of 0.02 mg/dm^2 corresponding to < 0.120 mg/kg food. The mean of three replicates with 3% acetic acid for 2 h at 70 °C, 2 h at 100 °C, and 10 days at 40 °C were 0.190, 0.950, and 0.760 mg/kg food, respectively. Comparatively, the mean of three extractions with 10% ethanol for 2 h at 70 °C, 2 h at 100 °C, and 10 days at 40 °C were 0.320, 0.610, and 0.260 mg/kg food (Knuppe, 2005). All extractions were considerably (i.e., more than an order of magnitude) below the lowest SML presented in this manuscript or stipulated by EU (2011).

13. Discussion

POM, a polymer composed of FA, is used inter alia for kitchenware and food processing machines for multiple use. FA may migrate from POM into food and thereby may lead to exposure of consumers. FA is classified as a Category 2 mutagen and Category 1 B carcinogen based on the assessment of RAC (2012). The decision on carcinogenicity relied on local tumor induction in the nose of rats after inhalation. As discussed in many comprehensive reviews, there are strong indications that the carcinogenic effect has a threshold given by local cytotoxicity and subsequent regenerative cell proliferation. Although the largest dataset for carcinogenicity is available for the inhalation exposure route, the classification for carcinogenicity was not restricted to this route because there are no valid data for the dermal route and contradictory evidence for the oral route, i.e. papillomas described in the forestomach by Takahashi et al. (1986) that were not noted in the most valid 2-year carcinogenicity study of Til et al. (1989). With this classification of FA it seemed timely and necessary to derive safe exposure levels and a SML for FA in food. To this end, all repeated oral dose studies published within the last decades not only for mammals but also for birds have been assessed here for the first time.

Presently, total migration of FA into food is regulated by a SML of 15 mg/kg food in EU (2011). In the past, several agencies and

committees have proposed safe oral exposure levels starting from the NOAEL for local irritation in the stomach of the Til et al. (1989) long-term study, and applying the standard UF of 100. An example is WHO (2005, 2011) with a tolerable concentration in drinking water of 2.6 mg/l. At first sight it seems plausible that the toxicological profile of FA is governed by local irritation because of its rapid detoxification and systemic effects may not to be expected as supported by a large number of inhalation studies. But this may not necessarily be the case for oral studies in which administered doses may be a factor of > 10 higher than in the inhalation studies. Besides to the well-known local irritation, the review of literature indicated to the kidney of adult experimental mammals and the gonads (and leukocyte counts) of juvenile birds as potential targets of systemic toxicity, target organs that up to now have not been taken into consideration. Under such conditions the first metabolite of FA, namely formic acid, may come into play. As potential long-term consequences of mild, non-life threatening chronic acidosis are unknown and determination of blood pH does not belong to the standard toxicological repertoire, a conservative derivation of safe exposure levels has to consider such a possibility.

Derivation of safe exposure levels is a 2-step process. First the PoD has to be defined that generally is the NOAEL for the appropriate route of exposure. Data after oral exposure via drinking water or feed is to be used for definition of a SML. Due to the high reactivity of FA, studies with application in drinking water give the most straight forward NOAEL. A slightly different situation exist for feeding studies as used in studies with birds or dogs. Under such conditions it must be taken into consideration that FA may bind to protein or other constituents in feed depending on many variables (e.g. time and temperature of storage). As the FA/feed mixtures in such studies were not analyzed for free FA, the extent of volatilization or (irreversible) binding to feed components and, thus, the actual FA dose is unknown. In addition, as noticed by Kowalczyk et al. (1993), such binding to protein may lower the nutritive value of feed, a potential confounder inherent to such experiments. Such an effect may also come into play if high FA concentrations in drinking water come into contact with feed adding to the observed reduction of body weight in such studies. The uncertainties for definition of the exact amounts of FA at the NOAEL in feeding studies are balanced by the fact that the low concentrations of FA at the SML may also volatilize from food or bind to food constituents, albeit to an unknown extent. In spite of these uncertainties, nominal dose levels for all feeding studies are given here in mg/kg feed.

In the 2-year study of Til et al. (1989) effects were observed indicating to kidney toxicity. However, these were interpreted by the authors as secondary due to massive decrease of water intake, but the reference given (Elliott, 1986) does not support this assumption. Some indications for kidney toxicity were also observed in a study with cockerels after application via feed or directly into the crop. In addition, findings in birds exposed before the age of sexual maturity suggested an adverse effect on the testes and at higher dose levels also female sex function seemed to be affected. Similar observations were not recorded in mammalian species. However, testicular effects noted in birds before sexual maturity have no counterpart in dietary or gavage mammalian toxicity studies that always started with young adult animals. As a consequence, conservative derivations of safe exposure levels should not only be based on local but also on systemic effects, e.g. on the kidney in adult animals and male sexual function before maturity.

Table 6
Migration studies with POM (Knuppe, 2005).

	Migration expressed as	
Migration condition	mg/kg food	mg/dm^2
Acetic acid 3%, 2 h, 70 °C	0.190	0.032
Acetic acid 3%, 2 h, 100 °C	0.950	0.158
Acetic acid 3%, 10 d, 40 °C	0.760	0.127
Ethanol 10%, 2 h, 70 °C	0.320	0.053
Ethanol 10%, 2 h, 100 °C	0.610	0.102
Ethanol 10%, 10 d, 40 °C	0.260	0.043
Olive oil, 2 h, 70 °C	< 0.120	< 0.020
Olive oil, 2 h, 100 °C	< 0.120	< 0.020
Olive oil, 10 d, 40 °C	< 0.120	< 0.020

Each value represents the mean of 3 replicates.
d = days; h = hours.

Critical for derivation of safe exposure levels is the selection of appropriate UFs. Those of EFSA (2012) are taken as the basis. A specific situation exists for FA with its extremely rapid and highly conserved metabolism to formic acid as the first step. Formic acid itself is a physiological intermediate in the C1 pathway that may lead at higher concentrations to acidosis similar in all species. Therefore, major inter- or intraspecies variability in toxicodynamics or –kinetics are not to be expected including the toxicological consequences of acidosis. Such general considerations would per se justify reductions of the default UFs. But as no reliable quantitative data could be identified to justify modifications of the standard subfactors, the default UFs were generally retained. There was only one exception: in monkeys a massive depletion of folic acid, the major factor for formic acid metabolism, resulted in a clear increase of the half-life of formic acid by a factor of 2. This was the basis to reduce the toxicokinetic subfactor for intrahuman variability from 3.16 to 2. But as for all other subfactors the default values of EFSA (2014a) were used, the total UFs applied here are certainly sufficiently conservative.

The differences between the safe exposure levels derived for local irritation (32.5 mg/kg food), effects on testes and leukocyte counts in juvenile birds (21.5 mg/kg food) and on the kidney in rats (16.5 mg/kg food) deserve some critical comparison. The assessments for systemic effects were mainly based on the assumption that after metabolism of FA, formic acid and acidosis may become a driver for toxicity taking account of data from Malorny at al. (1965) after a single oral dose of FA. Although the dose level of the Malorney study is comparable to those of the oral repeated dose studies, the different biokinetics must be taken into consideration. A bolus dose is more likely to momentarily overwhelm the capacity to detoxify formic acid than after protracted dosing. Therefore, the SMLs derived for systemic effects entail an exaggeration for systemic toxicity based on the assumption of uncompensated acidosis after oral dosing and should be regarded as potentially overly conservative giving a lower bound for the SML. Under these considerations the SML derived for local effects seems to better reflect actual risks. This is further substantiated by the high endogenous degradation of FA estimated by EFSA (2014a) to be between 878 and 1310 mg/kg bw/d for adults. In comparison, the LOAEL of Til et al. (1989) is by a factor of ~10 lower. Whether such relatively low exposures compared to the endogenous turn-over may disturb the intermediary formic acid concentration is difficult to decide, but not very likely. The LOAELs in the bird studies are difficult to calculate in mg/kg bw/d because of substantial increases of body weight during the first weeks of exposure. Generally their LOAELs related to body weight are comparable to those of Til et al. (1989). A further critical annotation is necessary for all avian studies. Their interpretation often is hampered because there is no overlap between the endpoints studied within each of these investigations and comparable study designs are missing. Finally, it should be mentioned that formalin was used as source of FA containing about 15% methanol. As methanol may add after metabolism to the internal body burden of FA and formic acid, this was accounted for by adding 10% to the dose of FA.

The comparison of systemic NOAELs and LOAELs with the endogenous turn-over of FA is a strong argument that any systemic effects either on kidneys or testes are not driven by FA per se but rather by its first step metabolite, formic acid with its ensuing acidosis. Even if minute amounts of FA after oral exposure reach systemic circulation, its rapid degradation with the very short half-life will prevent a direct action of FA.

All safe exposure levels derived for local (32 mg/kg food) and

systemic toxicity (16.5 for the kidney and 21.5 mg/kg food for testes) exceed the total SML(T) of EU (2011) of 15 mg/kg food showing that the SML(T) is sufficiently conservative. Although the SML(T) is below the estimate of EFSA (2014a) that oral exposure to FA in humans from dietary sources is unlikely to exceed 100 mg/adult/d, it is clearly higher than the refined estimate for all natural food items, i.e. 4.1 mg/adult/d (EFSA, 2014b). Based on an assumed daily food consumption of 1 kg/adult/d, the body burden at the lowest SML derived here (16.5 mg/kg food) is by a factor of about 3700–5500 lower than the internal turnover of FA of 61440–91680 mg/adult/d (EFSA, 2014a), demonstrating the conservative nature of the approach taken in this manuscript. Finally, the highest migration of FA from POM of 0.95 mg/kg food after extraction with 3% acetic acid at 100 °C over 2 h is much lower than the lowest SML.

In summary, three different SMLs were derived, based on local effects in the stomach, systemic effects in the kidney of adult rats and in testes and blood (i.e., leukocyte counts) of immature birds. Preference should be given to the derivation relying on local effects while those for systemic effects can be regarded as conservative lower bounds. It is recognized that the assessment based on the bird studies is surrounded by uncertainties and birds are not a recognized model for assessing mammalian toxicity. However, the bird studies do provide supporting information for some of the effects observed in the rat studies. The most conservative SML derived in this manuscript is based on potential systemic effects in the kidney, while that derived from point-of-contact gastrointestinal cytotoxicity, irritation, and/or ulceration is by a factor of 2 higher. As such, the proposed SML is protective of all effects, including potential carcinogenicity related to cytotoxicity and regenerative hyperplasia at the point-of-contact. Our approach yielded values higher than the current SML(T) stipulated by EU (2011), showing the conservative nature of the latter. Comparison with experimentally determined migration from a representative sample of POM, demonstrated migration at least an order of magnitude below the lowest derived SML, indicating wide margins of exposure for residual formaldehyde in POM.

Conflicts of interest

Heinz-Peter Gelbke is a private consultants working for the POM task force. Ralf Eisert, Edgar Leibold and James Sherman work for companies that manufacture POM. Harrie Buist is employed by TNO Innovation for Life and TNO was compensated for his work by the POM task force. The authors have sole responsibility for the content and the writing of the paper. The interpretation and views expressed in the paper are not necessarily those of the author's employers.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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