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Introduction

The Threshold of Toxicological Concern (TTC) is a concept that is intended to establish an acceptable level of intake for an unstudied chemical that poses a negligible risk of carcinogenicity or toxic effects. It is a conservative estimate where the TD_{50}, or dose that causes 50% tumor incidence, is extrapolated to a dose that would be expected to cause a 1 in 10^6 incidence using available data. Certain structural groups have been identified to be of such high mutagenic potency that the TTC approach is not justified for these compounds. This group is comprised of aflatoxin-like-, N-nitroso, and alkyl-azoxy compounds and is referred to as the Cohort of Concern (CoC).

Regulatory guidance regarding the TTC and hence the CoC comes from several sources including the Food and Drug Administration (FDA)[1,2], the European Medicines Agency (EMA)[3], and the International Conference on Harmonization (ICH)[4]. The TTC approach has been used to evaluate impurities in pharmaceuticals, contaminants in consumer products and the environment, as well as biocompatibility in medical devices. The guidance documents establish identification threshold[5] for compounds that are not expected to increase tumor incidence. A key concern for biocompatibility testing is therefore: are components from the cohort of concern present at or above the ICH identification threshold. The following information is intended to assist in understanding the components in the CoC and their associated analytical challenges.

Cohort of Concern Background

1. Aflatoxins

Aflatoxins are a family of mycotoxins produced by *Aspergillus flavus*, *Aspergillus parasiticus*, and related fungi.[6] These mycotoxins are particularly toxic toward children and can lead to stunted growth, delayed development, liver damage, and liver cancer.[7–9] The fungi generally consume cereal grains, legumes, and tree nuts and thrive in hot and humid environments. Optimum growth conditions for *Aspergillus* are 37°C with 11.5%-14% moisture content.[6,10]

There are more than 14 different known chemical species in the aflatoxin family[11] (**Figure 1**) which includes the four listed below as well as several metabolites. Commonalities in the family include a five-membered ring system with an aromatic core. The root cause of toxicity is believed to be intercalation into DNA as well as alkylation of base pairs leading to mutation. While no level of aflatoxin has been shown to be safe for human consumption, action levels ranging from 20-300 ppb have been established for cereal-crops intended for use as animal feed[8] and significantly lower levels for direct exposure routes. Ingestion is the most common form of exposure however some members of the aflatoxin family can permeate the skin.[12] Aflatoxins have also been shown to bind to certain materials following exposure of the materials to actively-growing fungal colonies.

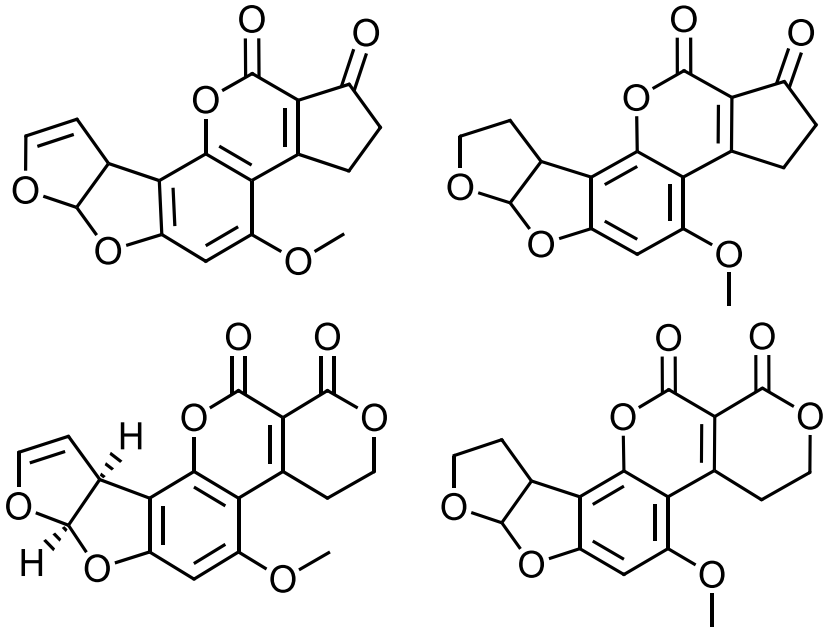

Figure 1: There are more than 14 different known chemical species in the aflatoxin family. Above are four of the most common. **Top Left:** Aflatoxin B_1**, Top Right:** Aflatoxin B_2, **Bottom Left:** Aflatoxin G_1, **Bottom Right:** Aflatoxin G_2.

2. *N*-nitroso Compounds (Nitrosamines)

Nitrosamines are compounds with the chemical structure $R^1N(-R^2)-N=O$ (**Figure 2**) where a nitroso group is bound to an amine. They are produced from the reaction of nitrites and secondary amines and have been shown to cause several types of cancer.[13] The formation of nitrosamines requires highly acidic conditions and/or high temperatures such as the digestive tract or a hot frying pan.

Significant levels of nitrosamines can be found in many foods preserved with nitrites such as cured meats and cheeses[14] as well as tobacco and e-cigarette vapor. Natural rubber has also been shown to contain quantities of nitrosamines.[15] Exposure can also occur through drinking water contaminated with nitrates such as agricultural runoff. On ingestion, the nitrates can be converted into nitrosamines by the acidic conditions of the stomach. The EPA has established 10 ppm as an action level for nitrosamines in drinking water[16] and an action level of 10 ppb in specific consumer items intended for children.[17]

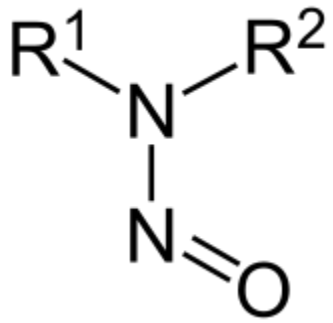

Figure 2: General structure of nitrosamine functional group.

3. Azoxy compounds

Azoxy compounds contain a functional group with the structure RN=N+(O-)R (**Figure 3**). They can be formed by the reduction of nitro compounds or by the oxidation of primary amines, both under a variety of conditions.[18] Specific conditions of concern include the exposure of primary amines like aniline to highly oxidizing conditions like hydrogen peroxide as well as exposure of aromatic nitro compounds to highly reducing conditions like alcoholic potassium hydroxide. Aliphatic amines like methylamine readily convert to azoxymethane following oxidation with *m*-chloroperbenzoic acid or oxone.[19] Aliphatic amines are frequently used as a potent carcinogen used to induce colon and intestinal cancers for murine studies. Because aliphatic amines can frequently be found in fermented seafood, wastewater, and human urine as a metabolite,[20–22] contact with these materials also carries associated risk for exposure to aliphatic azoxy compounds. Additional compounds that have been shown to directly convert into azoxy compounds in the body include alkyl hydrazines and alkyl azides.[19,23]

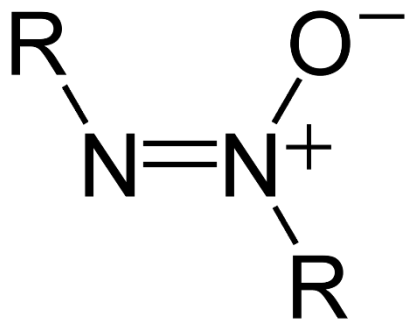

Figure 3: Structure of azoxy functional group.

4. Bibliography

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