Detection of Low Amounts of Sudan Dyes and other Illegal Dyes in Food and Oleoresins

Analytical Artefact or Cross-Contamination?

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Background

- **Synthetic Azo dyes (except Rhodamine B)**
- **General structure: \( R_1-N=N-R_2 \)**

![Chemical structures of Sudan I, Sudan II, Sudan III, Sudan IV, Para Red, and Rhodamine B](image)
Background

- Toxicology: Degradation products are considered to be carcinogens and teratogens (IARC, 1975/1978: Group 3)
  - Sudan I: Genotoxic and carcinogenic
  - Sudan II – IV, Para Red: Assumed to be potentially genotoxic and possibly carcinogenic because of structural similarities to Sudan I
  - Rhodamine B: Potentially genotoxic and carcinogenic
  - Orange II: Genotoxicity cannot be ruled out and the data on carcinogenicity are inadequate for any conclusion

- Insufficient data on any of the illegal dyes Sudan I-IV, Para Red, Rhodamine B, and Orange II to perform a full risk assessment
Background

- General applications:
  - Coloration of mineral products (e.g. diesel oil, fuel oil)
  - Coloration of wax products (e.g. shoe polish, candles)
  - Production of ball-point pen ink, felt pen ink

- Not authorized as food colors in the US or the EU (according to the European Parliament and Council Directive 94/36/EC)

- May 2003: European Authority reported finding of Sudan I at a level of 4,000 ppm in ground capsicums from India!
Background

- Since Mai 2003: several notifications via the Rapid Alert System for Food and Feed (RASFF):
  - Sudan I through Sudan IV in chilli powder, curry, turmeric, sumac, palm oil and processed products thereof
  - Para Red in chili and processed products thereof
  - Rhodamine B, Orange II in chili and turmeric
Background

- RASFF 2003, Notifications on chemical contaminants

- Spice from India: 22
- Sauces from India: 9
- Spices from Turkey: 9
- Sauces from Italy: 18
- Meat products from Italy: 7
- Spice from UK: 15
- Spices from the Netherlands: 5

Total: 75 notifications from 6 countries
Background

- **RASFF 2004, Notifications on chemical contaminants**

  - Palm oil from Ghana (Sudan IV): 53
  - Spices from India: 35
  - Spices from Turkey: 31
  - Spices from Pakistan: 10
  - Baked products from Italy: 14
  - Spices from Germany: 12
  - Sauces from Italy: 10
  - Spices from France: 7
  - Spices from the UK: 7
  - Spices from Italy: 7

Total 186 notifications from 8 countries
Background

- Countries of origin:
  - India, Turkey, Pakistan, Egypt (for raw spices)
  - Ghana, Nigeria, West Africa (for palm oil)

- Adulteration usually occurs during the milling of the dried pods
- Commonly analysed values: 0.1 – 100 ppm
  - However, levels of several 100 to 1,000 ppm of Sudan I are required to impact the visual appearance of chili powder!
Background

- In response to the adulteration, the EU issued
  - Decision 2003/460/EC requiring as a condition of import that all hot chili and hot chili products be tested for Sudan I
  - Decision 2004/92/EC to include Sudan II, III and IV
  - Decision 2005/402/EC to include turmeric and palm oil
Analysis

- Since 2003 some methods published utilizing GC-MS or HPLC with UV, DAD or MS detection
- HPLC-UV (e.g. ASTA 28.0) or DAD
  - LOQ = 500 – 1000 ppb (UV detection at 505 nm)
  - Not very specific
  - Possible interferences by carotenoids present in capsicums (also absorb in the range of some of the dyes)
- HPLC-MS/MS
  - LOQ = 10 - 100 ppb (signal suppression affects LOQ)
  - Possible spectral interferences
Scope of Analysis

Dyes found so far in food (reported in the EU RASFF):

- Sudan I – IV
- Para Red
- Orange II
- Rhodamine B
- Azorubin

➤ All mentioned dyes can be analyzed by HPLC-MS/MS within 1 run
Scope of Analysis

Dyes used illegally in countries from which spices originate and viewed as genotoxic and/or carcinogenic:

- Sudan Red 7B
- Methanil Yellow
- Auramine
- Butter Yellow
- Malachite/Leucomalachite Green
- Acid Red 73
- Congo Red
- Solvent Red I
- Naphthol Yellow
- Ponceau 3R
- Ponceau MX
- Oil Orange SS

➢ All the dyes can be analyzed by HPLC-MS/MS but not within 1 run!!
Results

- **Sudan I and Sudan IV in chili and turmeric (RASFF 2005)**

- **Frequently low amounts (< 1 ppm) of Sudan I and IV were found in chili and turmeric samples**
Results

- Illegal dyes in oleoresins

- Relevant dyes are Sudan I and especially Rhodamine B
- Analyzed levels are mainly below 100 ppb
Why are low concentrations (10 – 500 ppb) of illegal dyes found in raw materials like spices and oleoresins??

- No coloring effect!!
- False positive result??
- Present due to blending of adulterated commodities with non adulterated products??
- Cross-contamination from other sources, i.e. unintentional contamination??
Case study I

- Low amounts of Sudan I found in oleoresins (10 – 120 µg/kg)
- Supplier is audited, adulteration can be excluded
- **But:** Red colored lubricants are used for greasing of the extraction plants

- Analysis of the lubricant proved that this contains Sudan I in the ppm level!

Cross-contamination
Case study II

- Low amounts of Sudan IV were analyzed in paprika powder (10 – 20 ppb)
- Supplier is audited, adulteration can be excluded
- But: Usage of red bags for drying, transport and storage of the paprika pods
- Analysis of the red bags:
  - Sudan I in the ppm level
  - Sudan IV in the ppm level
  - Rhodamine B in the ppb level

Cross-contamination
Rhodamine B in oleoresins

- Rhodamine B can be found in the ink for the labeling on sacks
  - From there a cross-contamination can occur
- Capsicum oleoresins are found to be more often contaminated by Rhodamine B than chili oleoresins
- Does a contamination depend on different extraction procedures applied for chili, turmeric or capsicum oleoresins?
Conclusions

Possible sources for low amounts of illegal dyes in spices and oleoresins:

- Blending of contaminated with clean goods
- Analytical artifacts:
  - Carry-over after the analysis of highly contaminated commodities
  - Application of an insufficient selective detection
  - False interpretation of interfering peaks
- Cross-contamination during processing:
  - From red colored lubricants
  - From inks used for the inscription of sacks
  - Usage of red colored bags
European Standing Committee on the Food Chain and Animal Health, Section Toxicological Safety of the Food Chain, June 2006:

- **Reported findings of low levels (up to 200 ppb) of some illegal dyes**
  - Hypothesised to be present from other sources (e.g. inks used for labelling on sacks) and not from a fraudulent addition

- **In order to facilitate trade the following approach was discussed to be adopted across Europe:**
  - *Action Limit* of 500 ppb should be used for illegal dyes in raw materials like spices and palm oil
  - Approach should not be seen as Member States accepting adulteration
  - The food industry should continue to investigate sources of contamination when detecting levels below 500 ppb and take measures to reduce the levels where possible