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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

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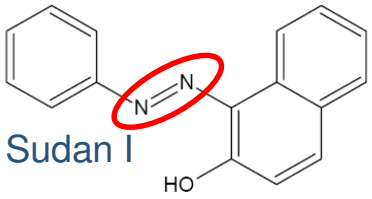
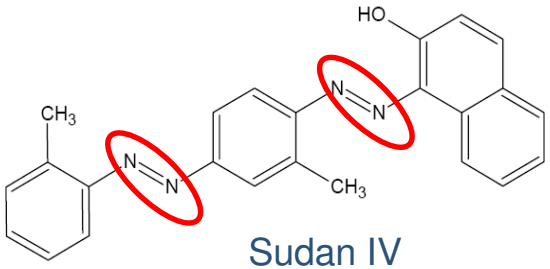
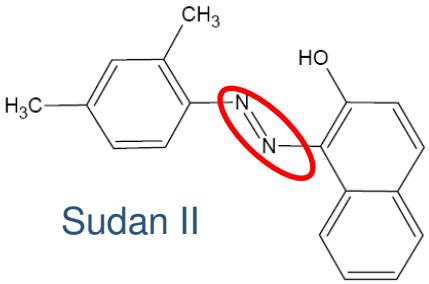
- **Synthetic Azo dyes (except Rhodamine B)**
- **General structure: R_1–N=N–R_2**



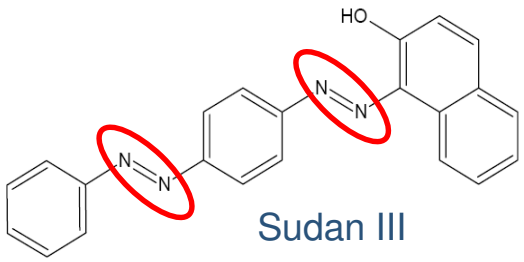
Sudan I



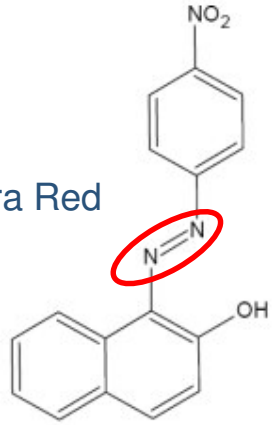
Sudan IV



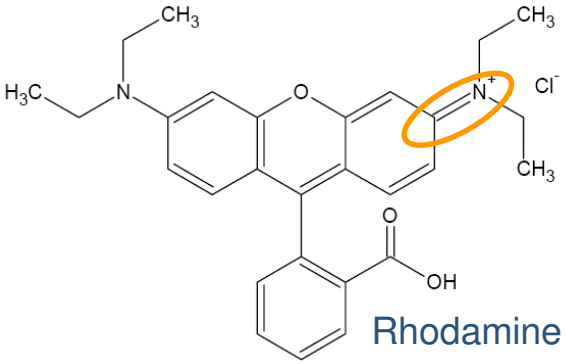
Sudan II



Sudan III



Para Red



Rhodamine B

Background

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- **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

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- 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

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

Background

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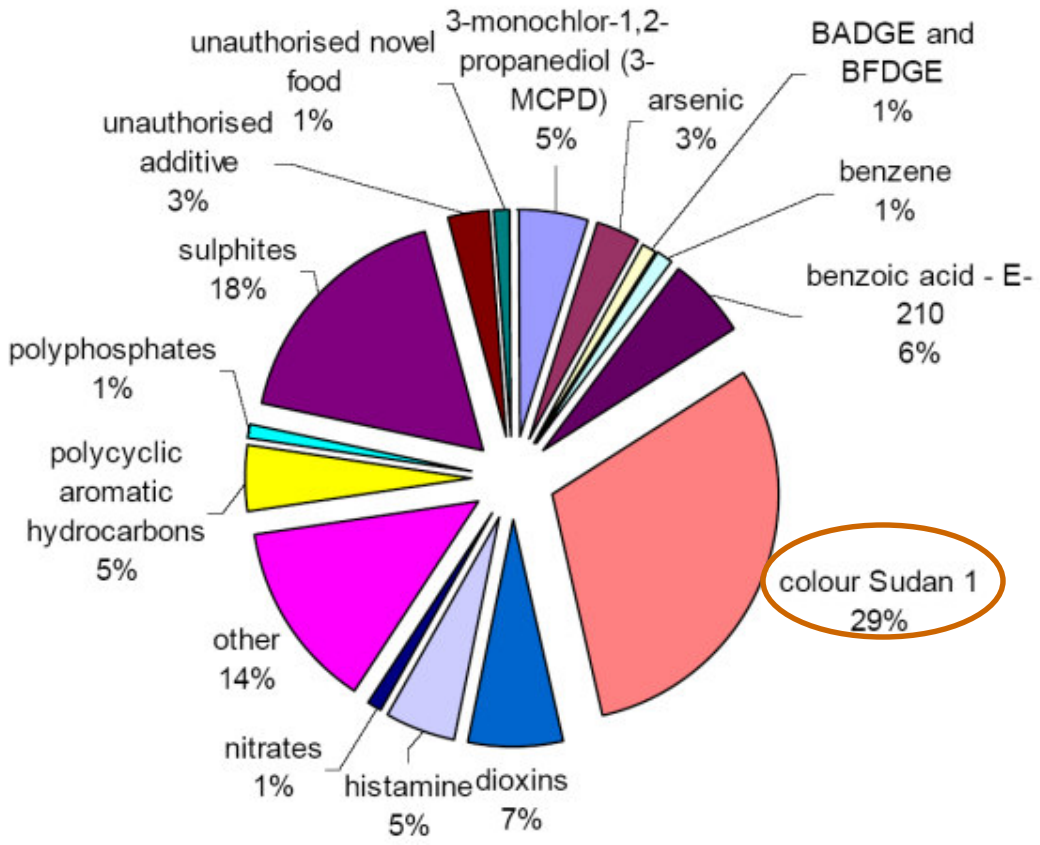
- **RASFF 2003, Notifications on chemical contaminants**



- **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

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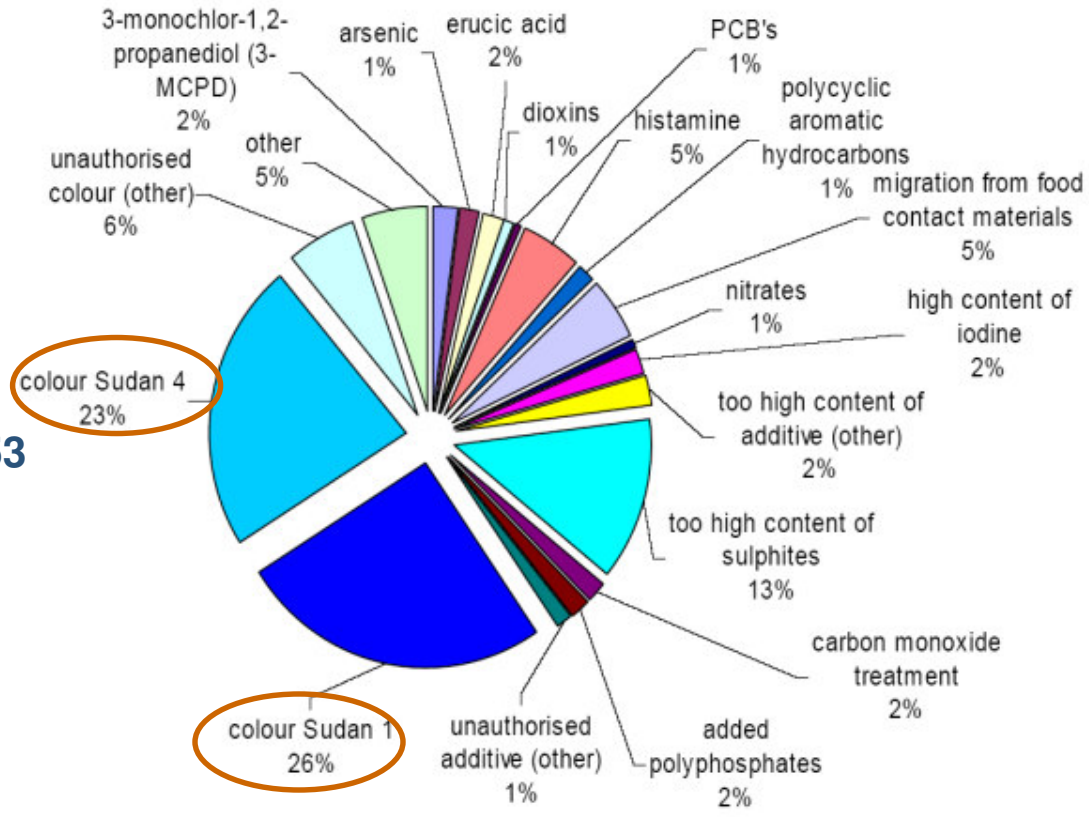
- **RASFF 2004, Notifications on chemical contaminants**



- 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

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- 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

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- 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

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- 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

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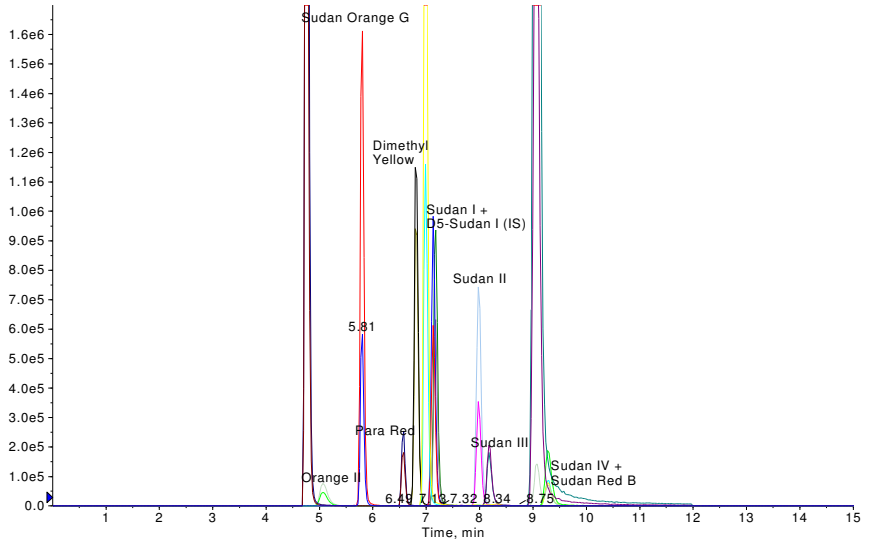
Dyes found so far in food (reported in the EU RASFF):

- 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

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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

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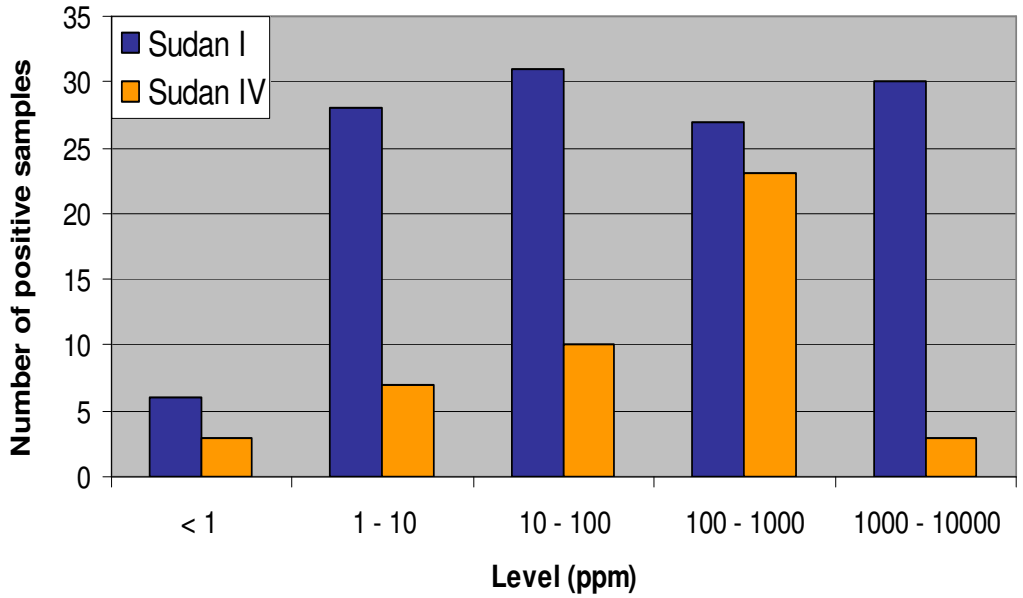
- **Sudan I and Sudan IV in chili and turmeric (RASFF 2005)**



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

Results

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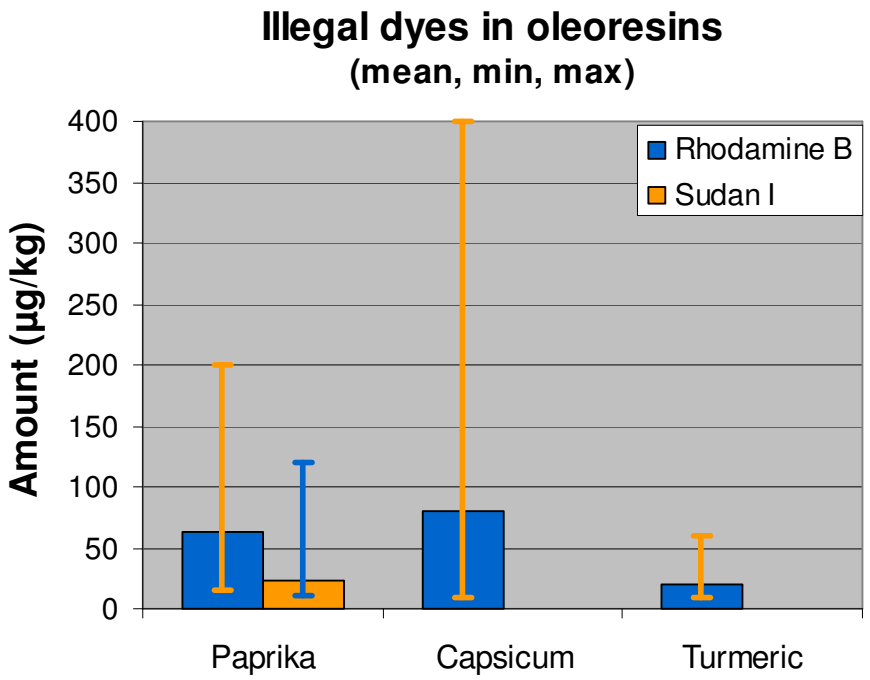
- **Illegal dyes in oleoresins**



- **Relevant dyes are Sudan I and especially Rhodamine B**
- **Analyzed levels are mainly below 100 ppb**

Results

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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

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- 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

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- 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

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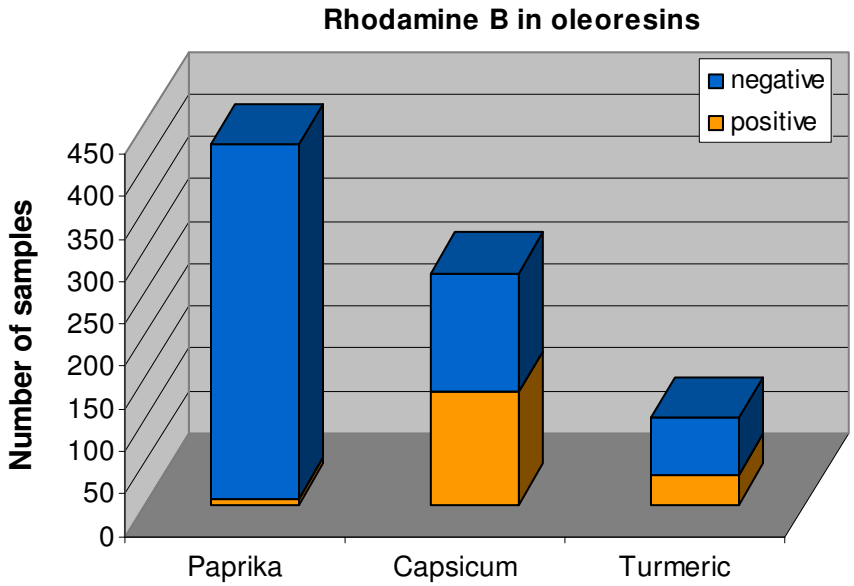

- Rhodamine B can be found in the ink for the labeling on sacks
  - From there a cross-contamination can occur
- Capsicum oleoresins are found to be more often contaminated by Rhodamine B than chili oleoresins
- Does a contamination depend on different extraction procedures applied for chili, turmeric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

Conclusions

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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**

Conclusions

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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**