



In-depth analysis of isolated fractions of printed samples

Mitglied bei:



AUSTRIAN COOPERATIVE RESEARCH

The data shown in this presentation were generated to follow up Ames positive effects in printed polyolefin recyclates.

The tested samples are not representative for the market and are partially of unknown origin.

No general safety concern of printing inks can be concluded based on this data.

National law on printing inks...

... highlights that mutagenic substances in printing inks are critical

German printing ink ordinance

The following substances groups are permitted to be used to manufacture printing inks:

Evaluated substances

- Printing ink ordinance contains a positive list of substances (BfR risk assessment)
- Substances on (EU) 10/2011 positive list (without any specific restrictions)

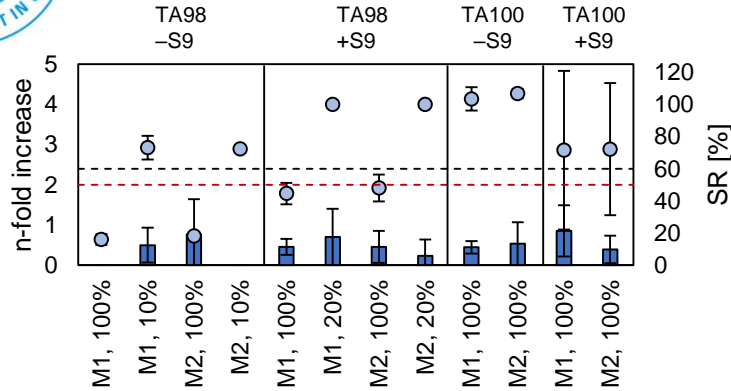
Non-evaluated substances

- Printed part not in direct food contact
- < 10 ppb (do not migrate)
- **No CMR substances**

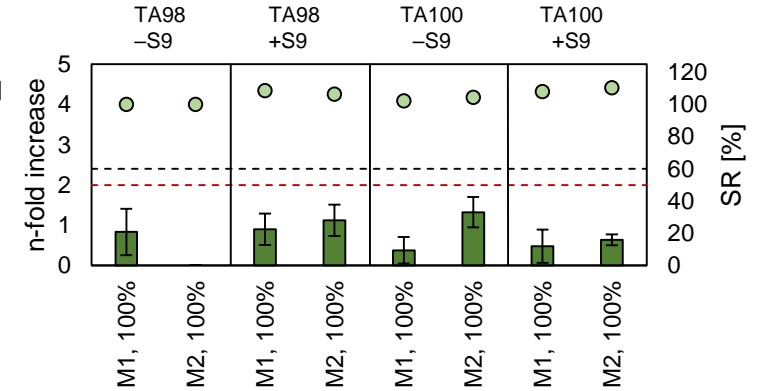
(same approach as “CH list B”)

Non-mutagenic printed FCMs

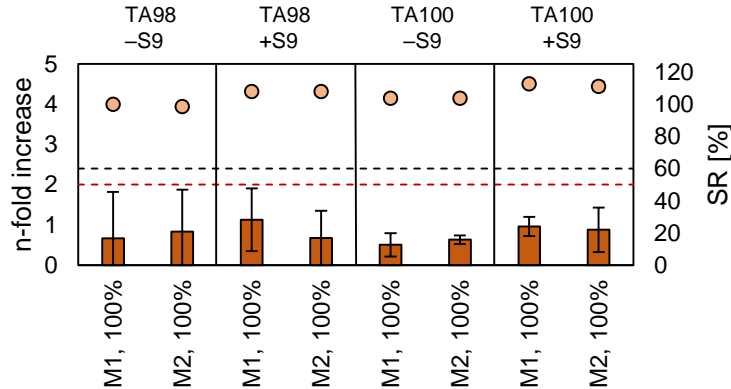
**Printed
PP bucket**



**Printed
PP foil**



**Printed
PP foil**



A portion of printed FCM samples scored non-mutagenic.

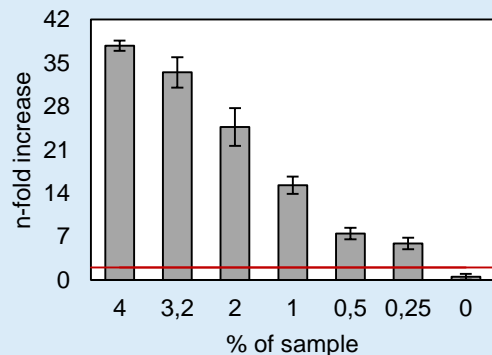
Partially, printing inks triggered inhibition.

Printed outside of coated paper scores Ames positive

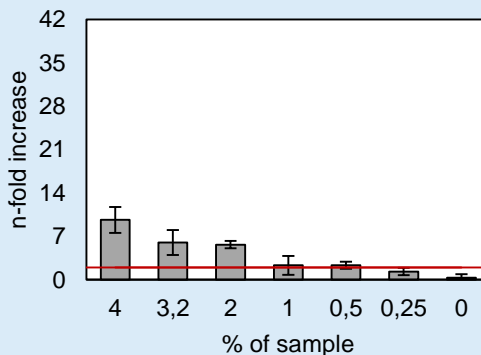
Printed outside
(Non food contact side)

Test condition: TA98 –S9

Coated paper 1



Coated paper 2



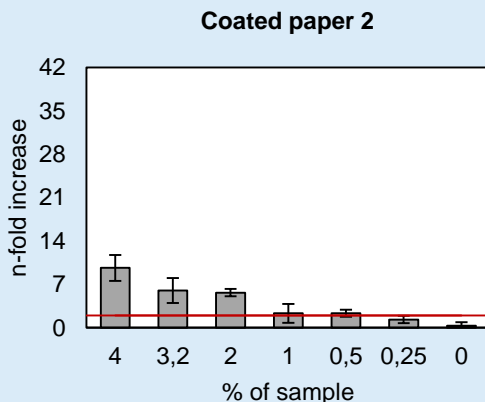
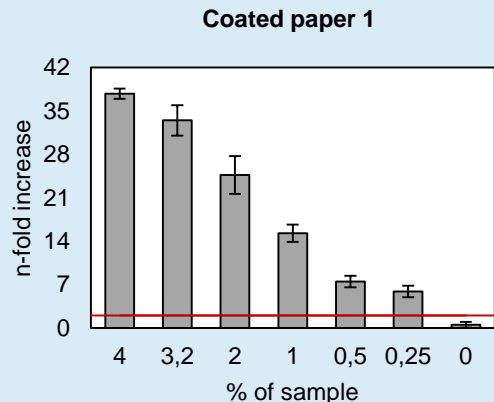
In-vitro mutagenicity

Printed outside of coated paper scores Ames positive

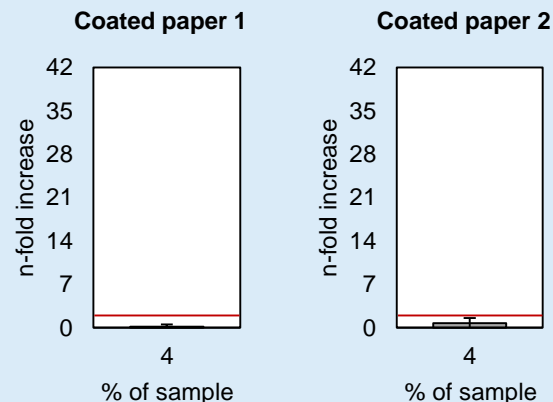
Printed outside
(Non food contact side)

Test condition: TA98 –S9

Non-printed inside
(Food contact side)



In-vitro mutagenicity



No *in-vitro* mutagenicity

In-vitro mutagenicity still critical: (EU) 10/2011 - no CMR substances behind functional barrier
German Printing Inks Ordinance: use of mutagenic substance not allowed in printing inks!

Printing inks are a risk factor for mutagenic effects in recycling!

Unprinted foil

Printed foil

Recycling
process

Recycling
process

Granules

Granules

Printing inks are a risk factor for mutagenic effects in recycling!

Only **printed AND recycled** materials scored Ames positive!

Unprinted foil

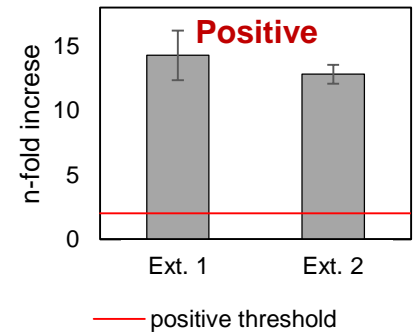
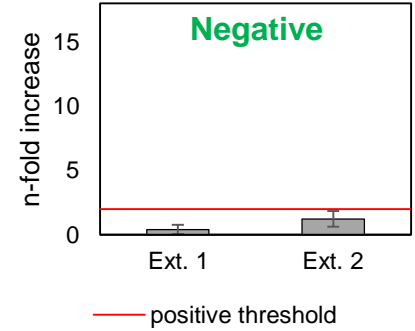
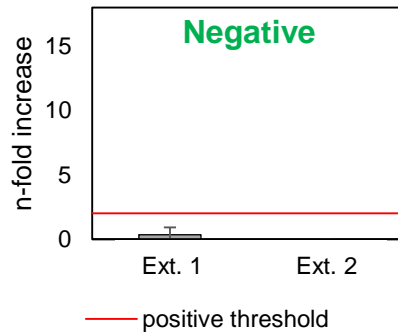
Printed foil

Recycling process

Recycling process

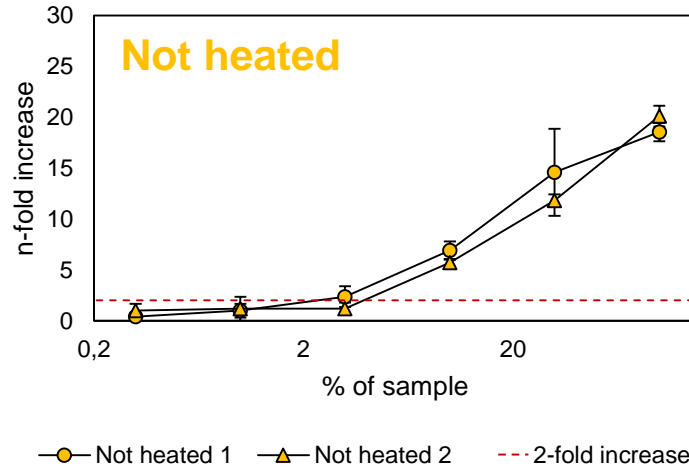
Granules

Granules



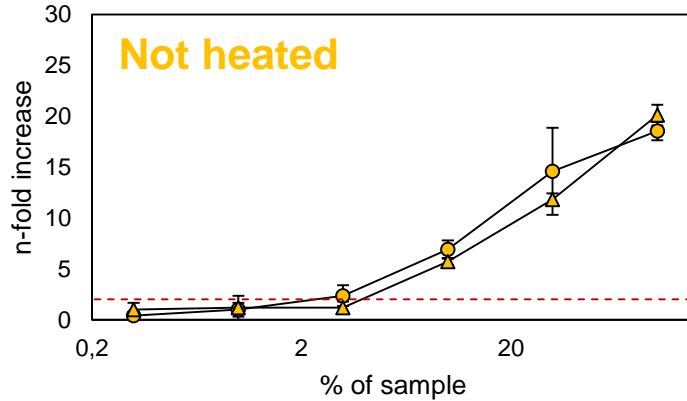
Heating increased mutagenic activity of printed FCM bag

Printed LDPE bag

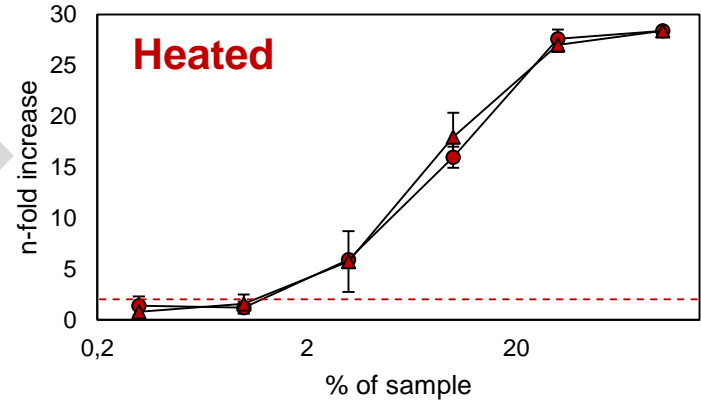


Heating increased mutagenic activity of printed FCM bag

Printed LDPE bag



Δ , 160°C,
3 min



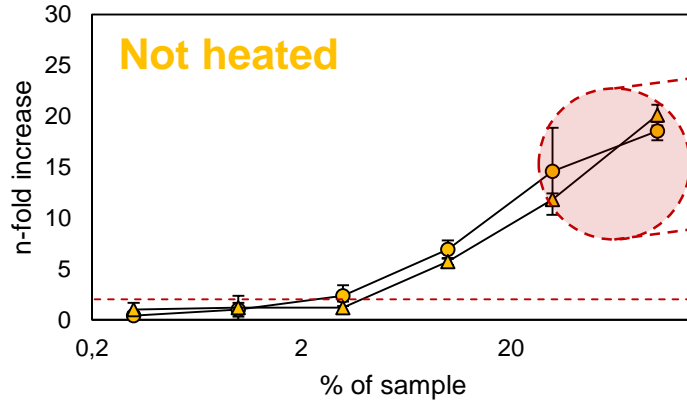
—●— Not heated 1 —▲— Not heated 2 - - - 2-fold increase

—●— Heated 1 —▲— Heated 2 - - - 2-fold increase

Untreated, printed LDPE bag scored already Ames positive.
A mutagenic response was triggered by heating (relevant for recyclability).

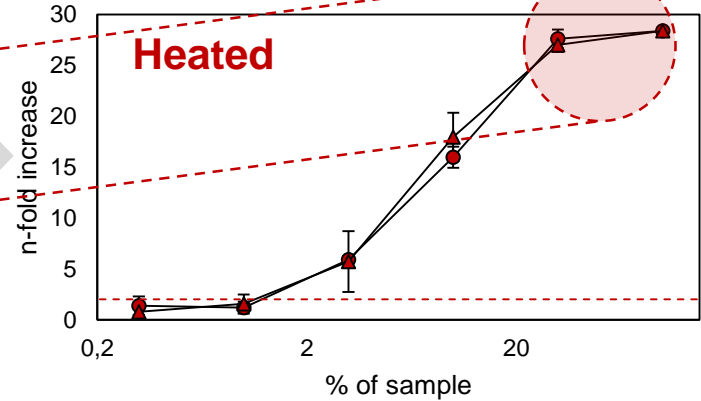
Heating increased mutagenic activity of printed FCM bag

Printed LDPE bag



● Not heated 1 ▲ Not heated 2 - - - 2-fold increase

**Δ, 160°C,
3 min**



● Heated 1 ▲ Heated 2 - - - 2-fold increase



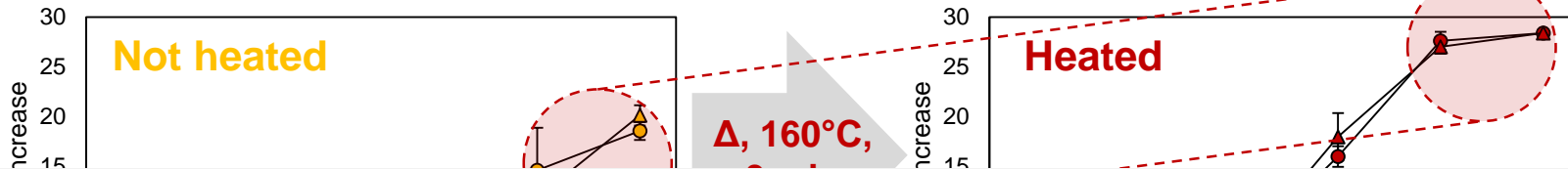
Untreated, printed LDPE bag scored already Ames positive.
A mutagenic response was triggered by heating (relevant for recyclability).

Potential sources for mutagenic activity in printed FCMs:

- Nitrocellulose binder
Source for nitroso compounds
- Azo dyes
Degradation of azo dyes is a source for (primary aromatic) amines
- Nitrosamines
Formation by decomposition of nitrocellulose inks if a secondary amine is present

Heating increased mutagenic activity of printed FCM bag

Printed LDPE bag



LDPE foil printed with: Nitrocellulose based printing inks

Possible explanation: Degradation products of nitrocellulose could trigger Ames positive outcome?

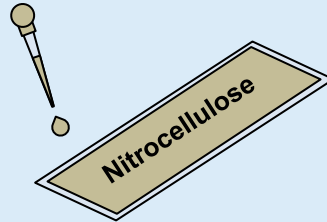
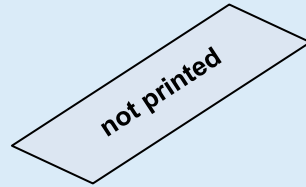


Untreated, printed LDPE bag scored already Ames positive.
A mutagenic response was triggered by heating (relevant for recyclability).

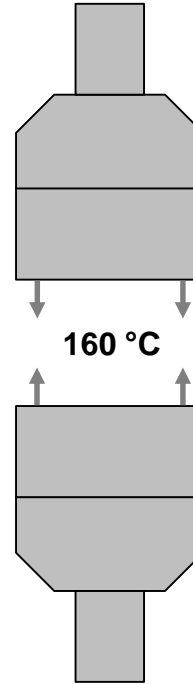
Preparation of printed reference materials



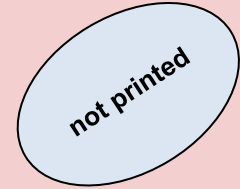
Not heated



Hydraulic press

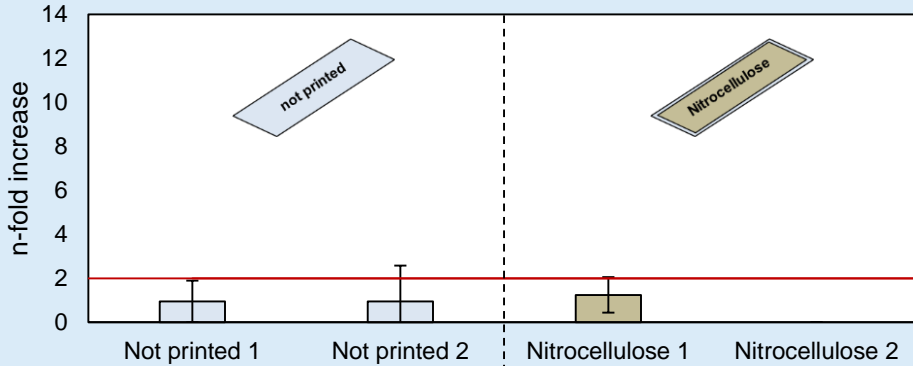


Heated



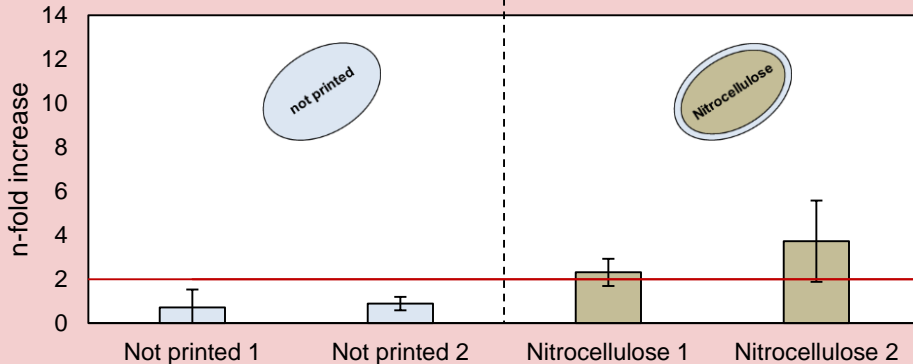
Heating of Nitrocellulose triggers slight mutagenic activity

Not heated



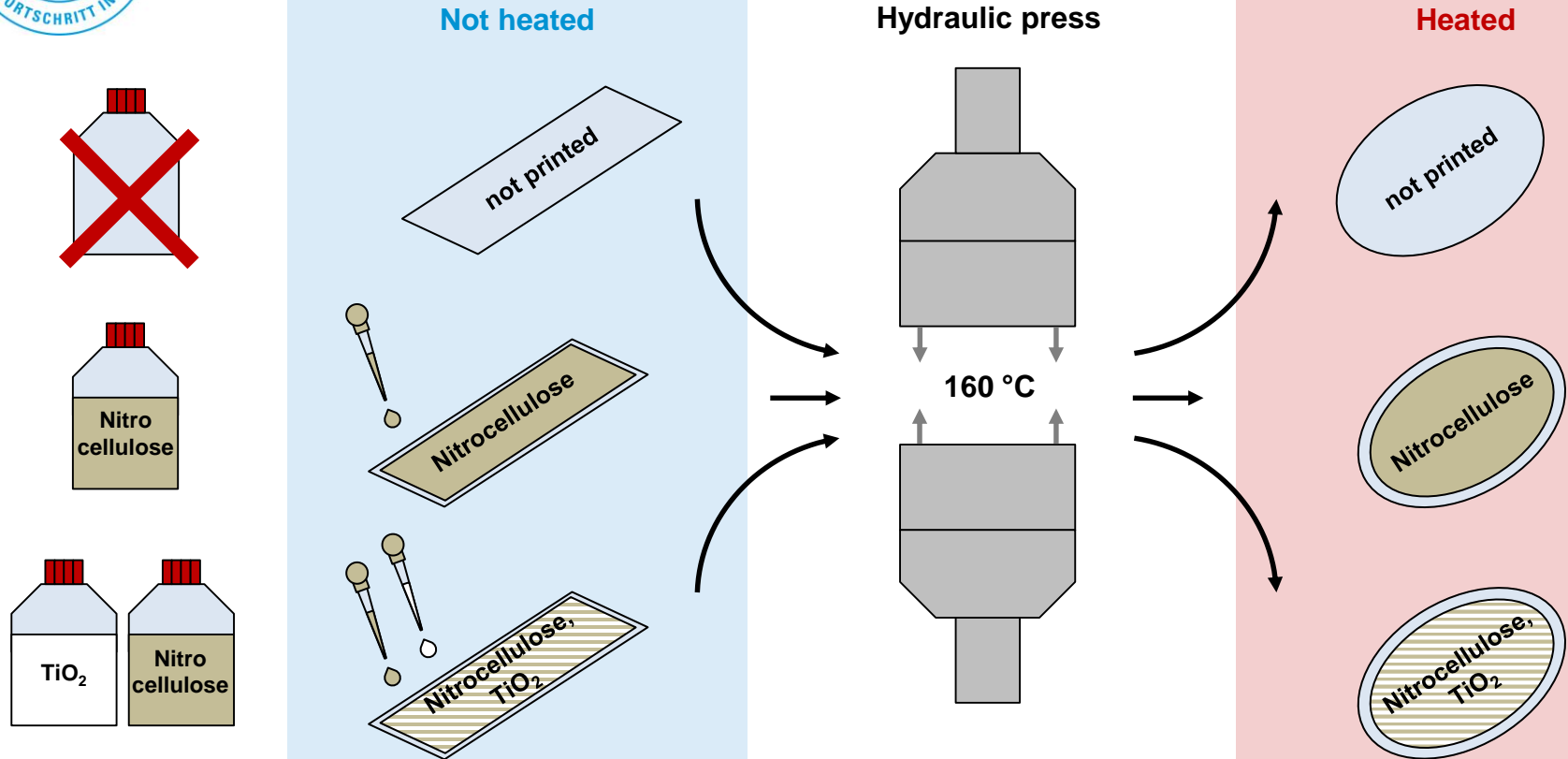
Could **inorganic catalytically active pigments** promote the formation of mutagenic activity?

Heated



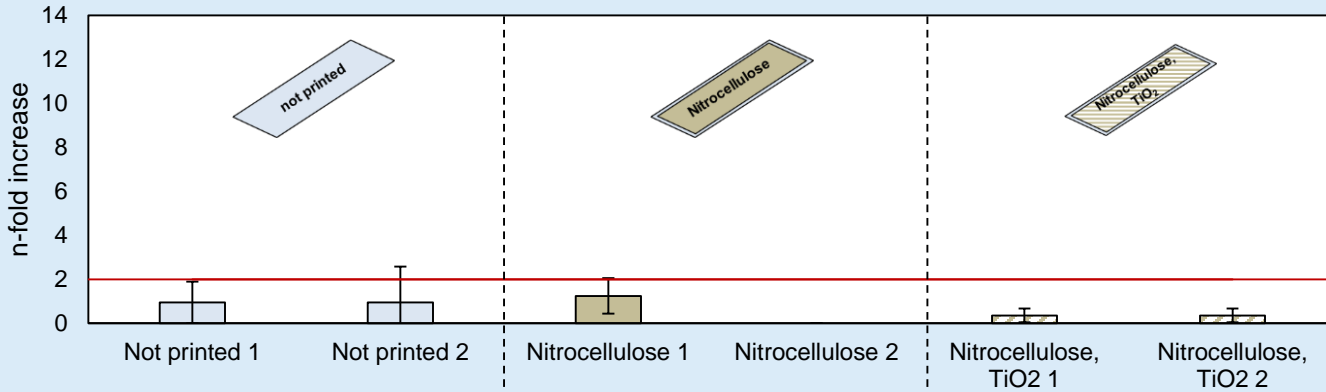
Titanium dioxide (TiO_2) is a widely used pigment with catalytic activity.

Preparation of printed reference materials

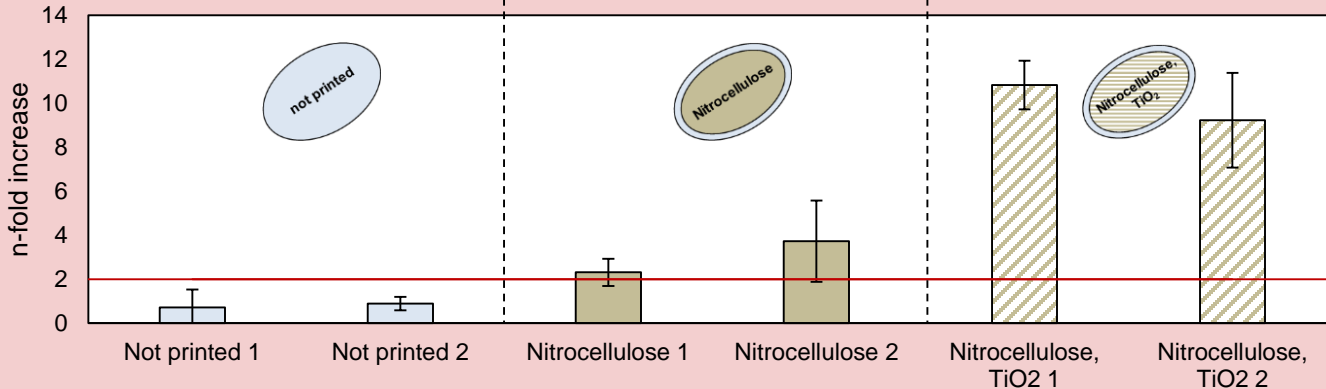


Heating of Nitrocellulose + TiO₂ triggers mutagenic activity

Not heated

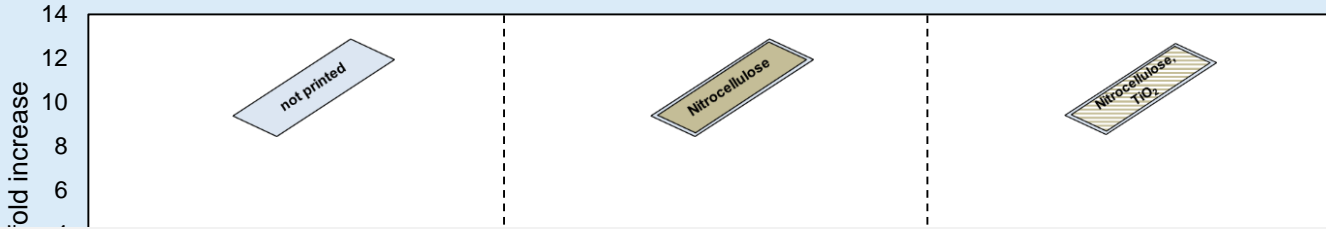


Heated



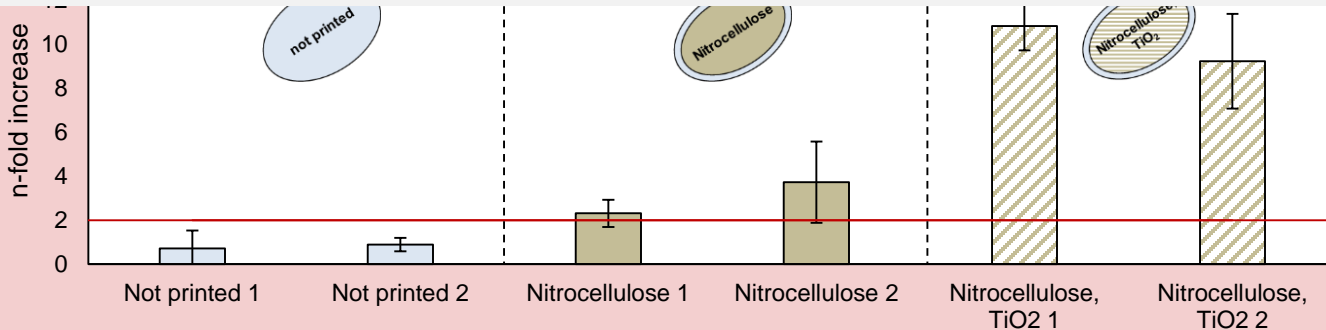
Heating of Nitrocellulose + TiO_2 triggers mutagenic activity

Not heated



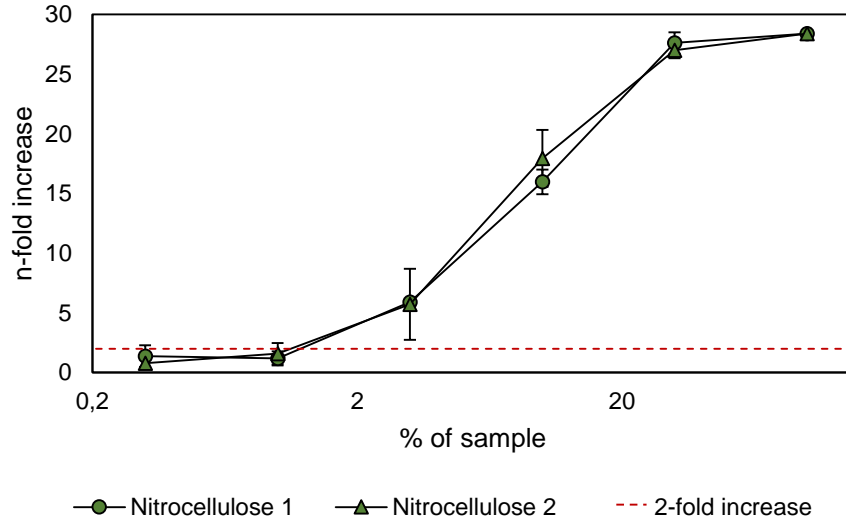
Heating of nitrocellulose triggers a mutagenic response in the Ames test.
Addition of TiO_2 increases the mutagenic effect, probably by a catalytic effect.

Heated

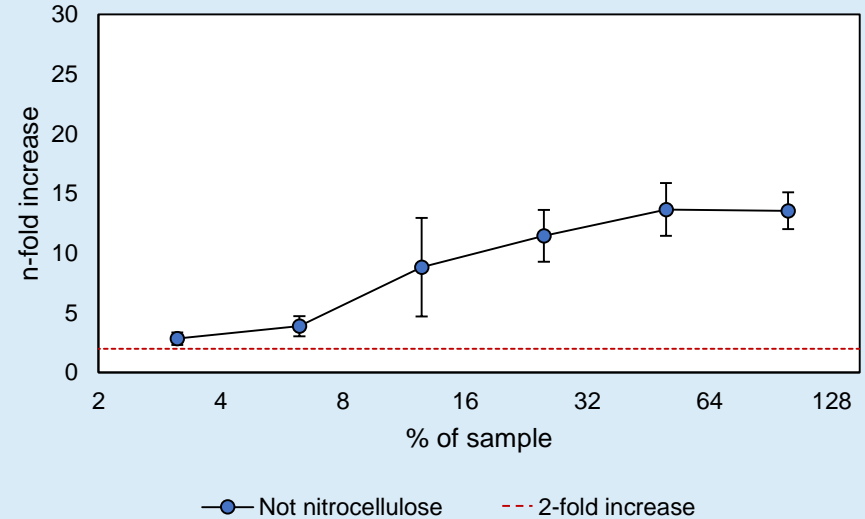


Nitrocellulose is not the only risk factor

Nitrocellulose binder



Non-Nitrocellulose binder



Nitrocellulose is not the only source for mutagenic activity.

Potential sources for mutagenic activity in printed FCMs:

- **Nitrocellulose binder**
Source for nitroso compounds
- **Azo dyes**
Degradation of azo dyes is a source for (primary aromatic) amines
- **Nitrosamines**
Formation by decomposition of nitrocellulose inks if a secondary amine is present

Potential sources for mutagenic activity in printed FCMs:

- ~~Nitrocellulose binder~~
Source for nitroso compounds
- Azo dyes
Degradation of azo dyes is a source for (primary aromatic) amines ???
- ~~Nitrosamines~~
Formation by decomposition of nitrocellulose inks if a secondary amine is present

Primary aromatic amines were detected

Screening for printing ink components (multi-analyte method):

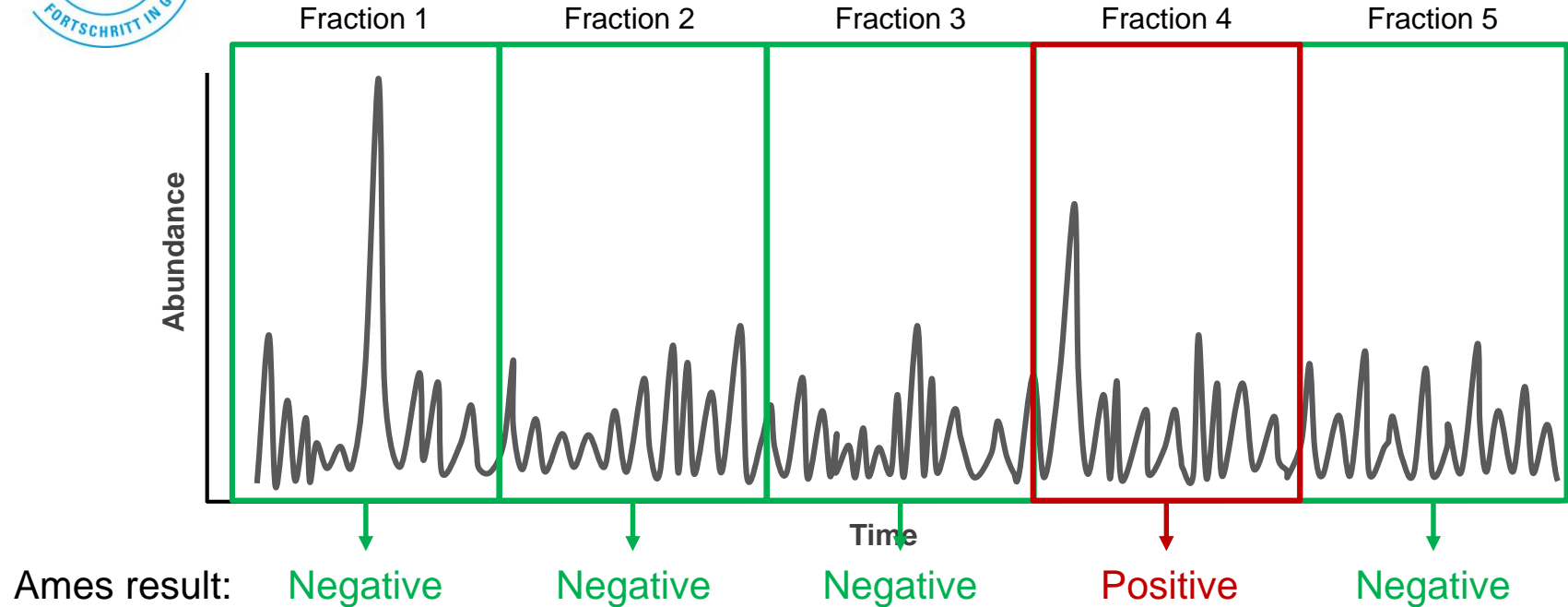
- Primary aromatic amines detected
- Concentrations > 2 ppb were found (limit for PAAs according to (EU) 10/2011)
- PAA concentration increased after heating / re-extrusion

Do PAAs explain Ames positive signals?

- PAAs mainly positive in TA100 +S9, printed samples positive in TA98 +S9
- PAA concentration does not explain the high activity in printed samples

Is there any other mutagenic substance group present?

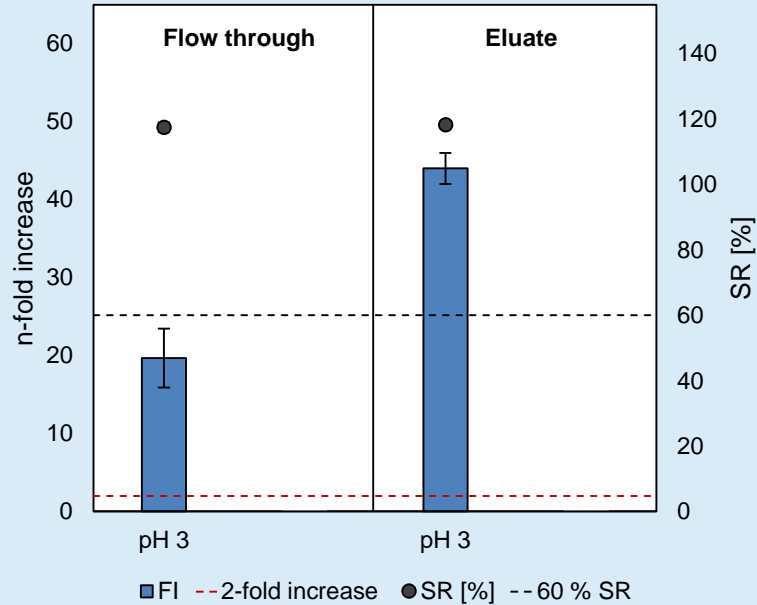
Fractionation: reduces the chemical complexity



Only the peaks in the Ames positive fraction need to be identified!

At least 2 mutagenic substance groups are present

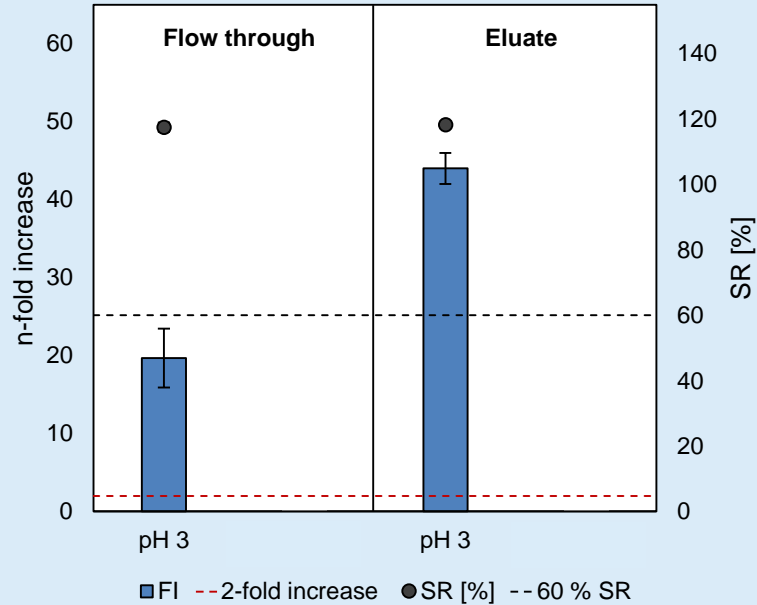
C18



≥ 2 different mutagenic substance groups?

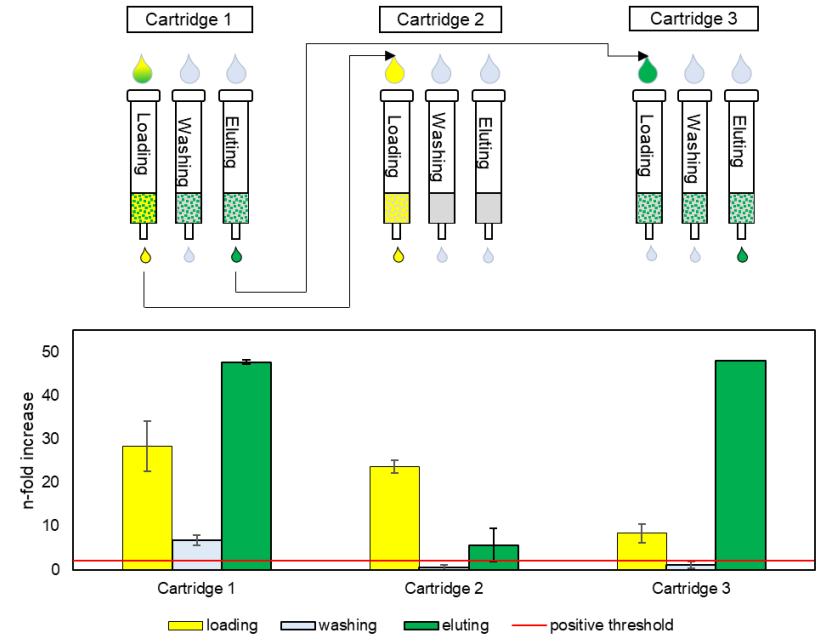
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C18

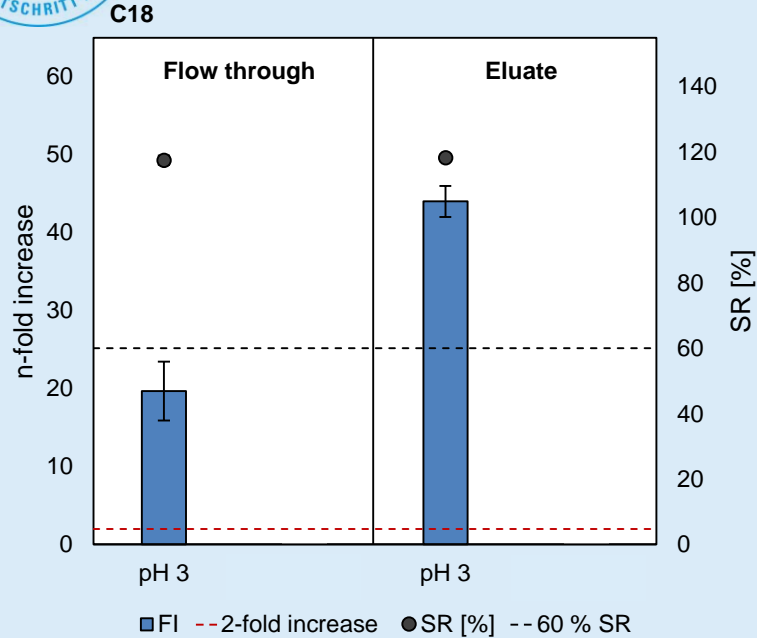


≥ 2 different mutagenic substance groups?

Confirmed by a follow up experiment:



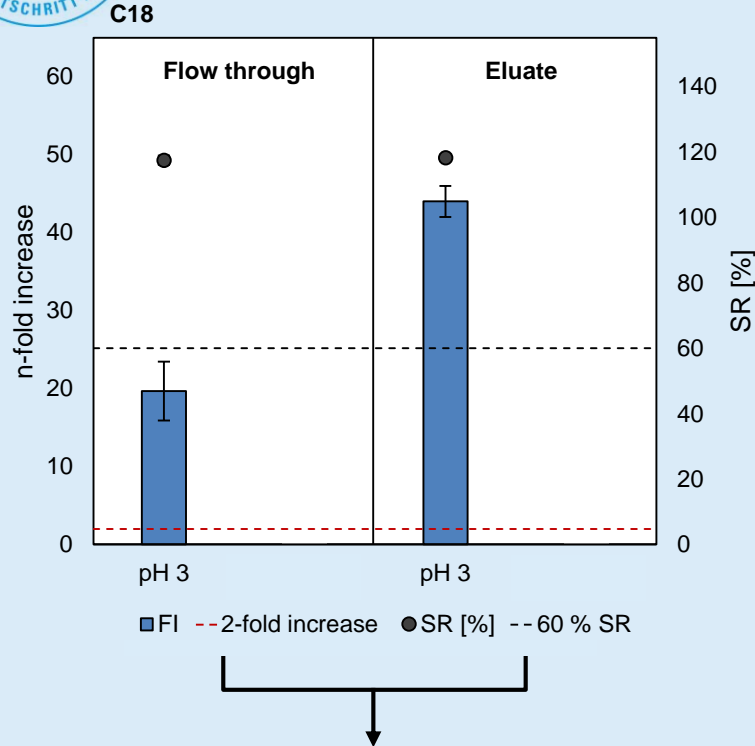
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≥ 2 different mutagenic substance groups?

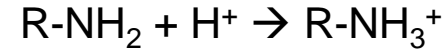


At least 2 mutagenic substance groups are present



Acidic pH (pH 3)

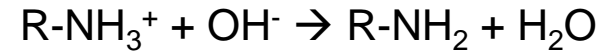
Amines are **positively charged**



- Do not stick to C18 Column
- Signal in flow through higher

Basic pH (pH 11.3)

Amines are **not charged**

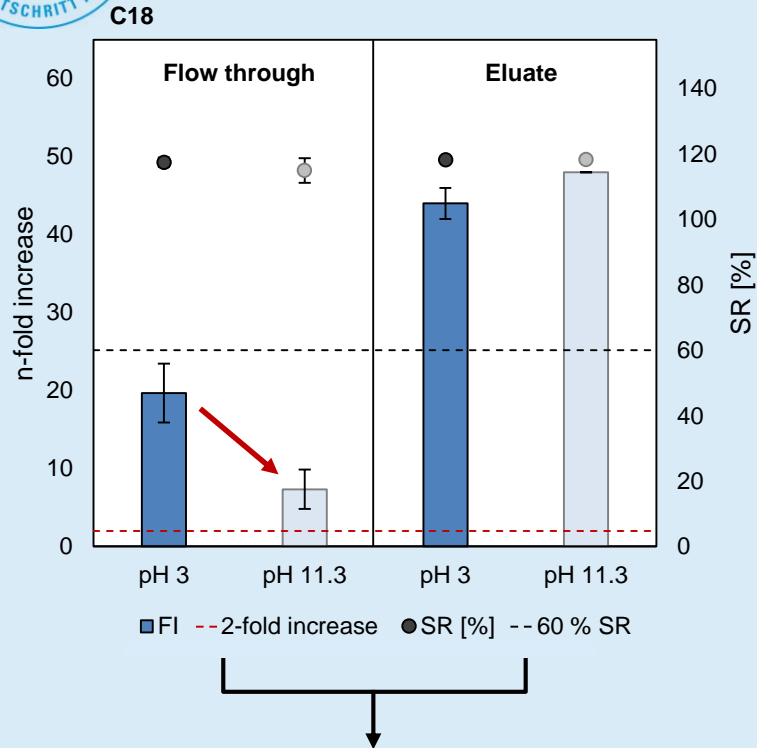


- Stick to C18 column
- Signal in flow through lower

≥ 2 different mutagenic substance groups?

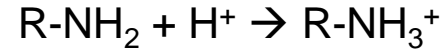


At least 2 mutagenic substance groups are present



Acidic pH (pH 3)

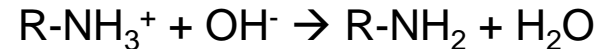
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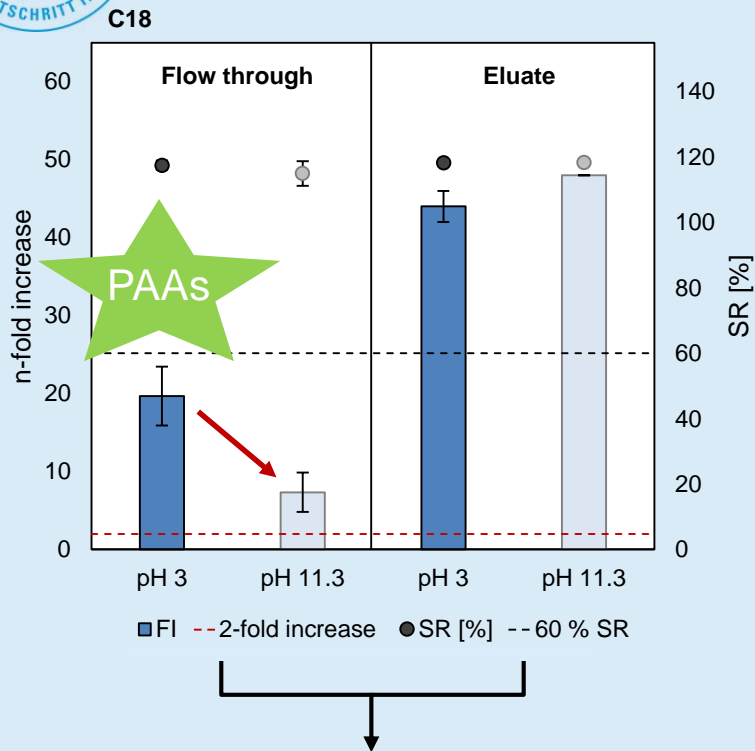
Basic pH (pH 11.3)

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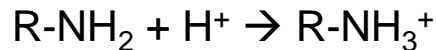
- Stick to C18 column
- Signal in flow through lower

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Acidic pH (pH 3)

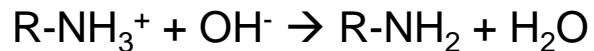
Amines are **positively charged**



- Do not stick to C18 Column
- Signal in flow through higher

Basic pH (pH 11.3)

Amines are **not charged**

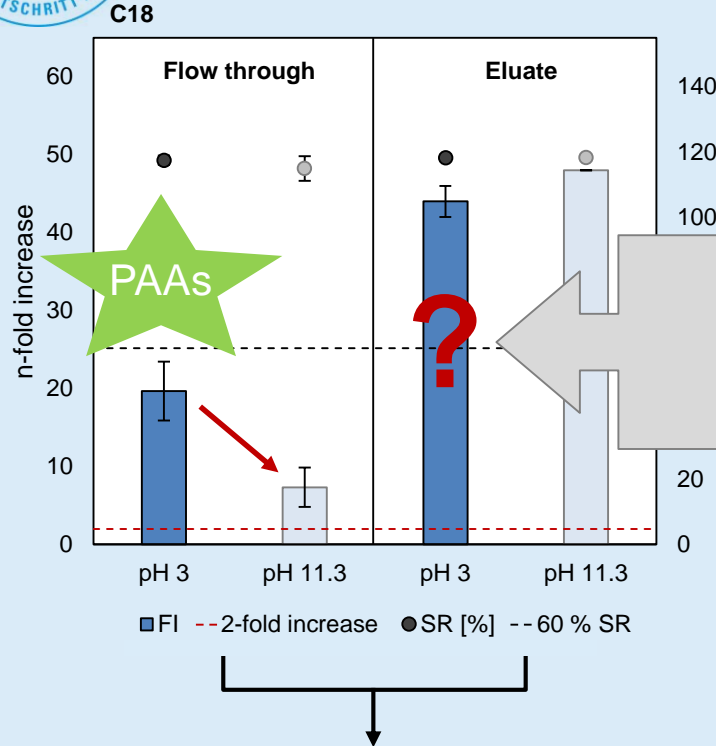


- Stick to C18 column
- Signal in flow through lower

≥ 2 different mutagenic substance groups?

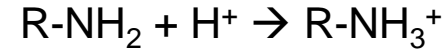


At least 2 mutagenic substance groups are present



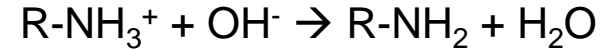
Acidic pH (pH 3)

Amines are **positively charged**



Identity of substances in eluate fraction - with stronger signals - remains unknown!

Amines are **not charged**



- Stick to C18 column
- Signal in flow through lower

≥ 2 different mutagenic substance groups?



However: Identification of the mutagenic source – not easy!

Standard GC-MS “NIAS”

Screening:

High number of peaks!

Source for mutagenicity could not be detected!

Hypothesis:

Mutagenic substance is probably **not volatile or semi-volatile**.

Probenname:	MeOH Extrakt	Polarität:	Positiv	Medium:	MeOH
C50H200N10O10S2P2					
Peakname	Summenformel	Score Summenformel	Masse (m/z)	Rt [Minuten]	Konzentration [ppb]
114.0908 / 3.26	C6H11NO	60	114.0908	3.3	135.0
177.0657 / 3.32	C9H8N2O2	90	177.0657	3.3	222.0
195.0875 / 3.92	C8H10N4O2	84	195.0875	3.9	199.0
177.0656 / 3.94	C9H8N2O2	65	177.0656	3.9	194.0
123.0626 / 3.94	No formula found	0	123.0626	4.0	157.0
122.0595 / 3.96	C7H7NO	52	122.0595	4.0	1209.0
135.0544 / 4.02	C7H6N2O	61	135.0544	4.0	149.0
151.0861 / 4.11	C8H10N2O	57	151.0861	4.1	202.0
120.0551 / 4.26	No formula found	0	120.0551	4.3	306.0
340.2594 / 4.35	C18H33N3O3	90	340.2594	4.3	118.0
151.0865 / 4.80	C8H10N2O	63	151.0865	4.8	459.0
160.0867 / 4.82	C9H9N3	84	160.0867	4.8	302.0
217.1072 / 5.02	C10H16O5	46	217.1072	5.0	162.0
323.1707 / 5.00	C14H26O8	83	323.1707	5.0	599.0
211.1437 / 5.24	C11H18N2O2	59	211.1437	5.2	148.0
453.3436 / 5.24	C24H44N4O4	71	453.3436	5.3	1419.0
275.1492 / 5.30	C13H22O6	80	275.1492	5.3	286.0
237.0756 / 5.34	C12H12O5	66	237.0757	5.4	169.0
246.1417 / 5.64	C6H15N9O2	84	246.1417	5.5	263.0
245.1294 / 5.90	C14H16N2O2	77	245.1294	5.5	1427.0
245.1386 / 5.47	C12H20O5	88	245.1386	5.5	1427.0
188.0930 / 5.56	C9H9N5	80	188.0930	5.6	324.0
281.1024 / 5.56	C14H16O6	55	281.1024	5.6	156.0
149.0706 / 5.58	C8H8N2O	43	149.0706	5.6	384.0
251.1855 / 5.58	C12H26O5	57	251.1855	5.6	644.0
207.0767 / 5.62	C10H10N2O3	59	207.0767	5.6	217.0
235.1904 / 5.64	C13H22N4	26	235.1904	5.6	128.0
180.1018 / 5.73	C10H13NO2	64	180.1018	5.7	220.0
228.1597 / 5.77	C12H21NO3	40	228.1597	5.8	185.0
566.4287 / 5.88	C30H55N5O5	87	566.4287	5.9	309.0
139.1114 / 5.93	No formula found	0	139.1114	5.9	134.0
193.0972 / 5.93	C10H12N2O2	52	193.0972	5.9	427.0
149.0225 / 6.06	C8H4O3	40	149.0225	6.1	171.0
237.0760 / 6.06	C12H12O5	53	237.0760	6.1	754.0
343.1395 / 6.06	C16H22O8	66	343.1395	6.1	2670.0
303.1805 / 6.06	C15H26O6	57	303.1805	6.1	221.0
195.1121 / 6.19	C10H14N2O2	53	195.1121	6.2	194.0

- Some printing inks could be a potential risk factor for mutagenic effects.
- Heating of nitrocellulose induced mutagenic activity - critical for plastic recycling!
- Besides nitrocellulose, printing inks provide additional – so far unknown – sources of mutagenic activity.
- A (small) fraction of mutagenic activity can be explained by primary aromatic amines.
- Most activity remains unexplained and cannot be detected by GC-MS screening.

To find source for mutagenic activity:

Defined printing inks / ink components will be systematically tested!



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