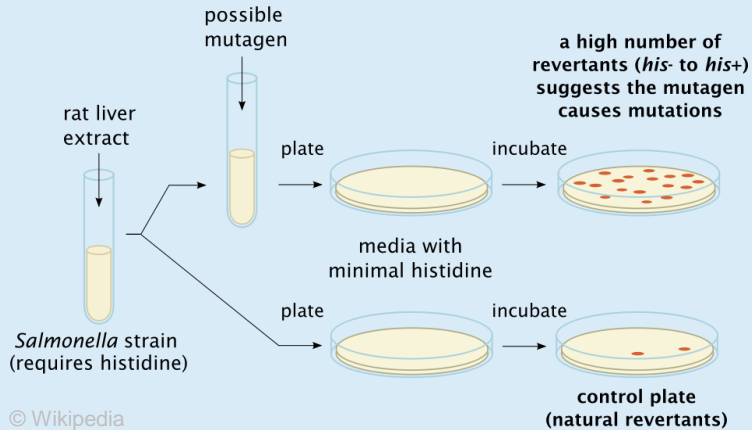


Lukas Prielinger & Christian Kirchnawy:

***In-vitro* screening results of the miniaturized  
Ames test on printed FCMs**

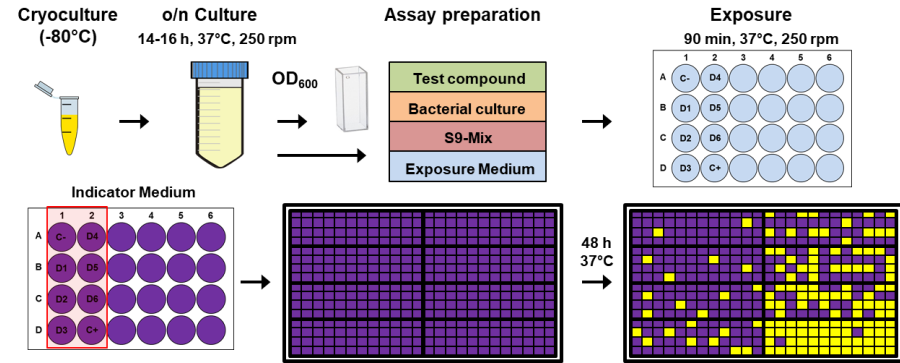
## Ames Test



© Wikipedia

- + Sensitive and robust
- + Broad acceptance (OECD Guidelines)
- + Focus on direct DNA-reactive substances
- Labour intensive
- Space consuming

## Miniaturized Ames Test (Ames MPF)

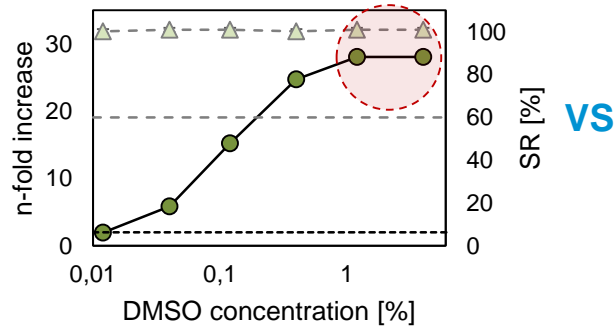


- Cultivation and exposure in liquid medium
- Colorimetric readout
- Higher throughput (microtiter plates)
- + Lower sample amounts required
- + Less incubator space required

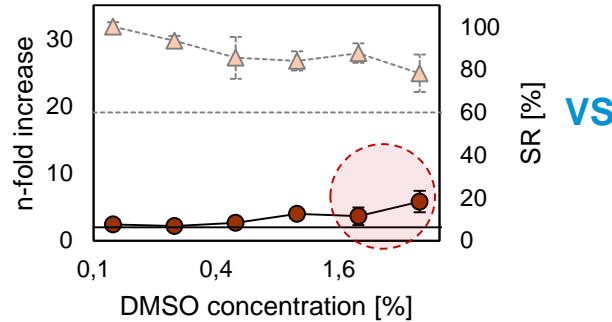
# Some polyolefin recyclates score strongly positive!

—●— n-fold increase    - - - - 2-fold increase    —○— SR [%]    - - - - 60% SR

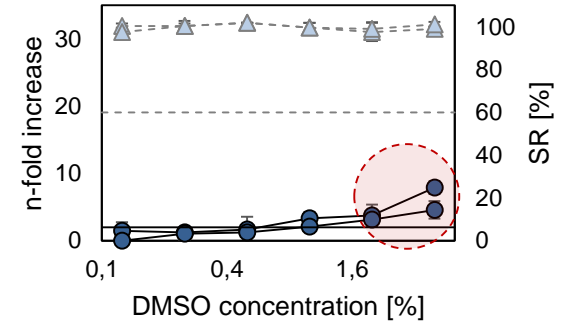
**Recycled polyolefin**



**Mutagenic reference oil  
(positive control)**



**Mutagenic reference sample  
(for inter-lab study)**



Some recycled polyolefins have strong positive effects in the Ames test, partially even higher than in mutagenic reference oils or intentionally spiked FCM samples.

However: Recycled PET scores negative in the Ames test.

# Degradation products of printing inks as a risk factor for recycling

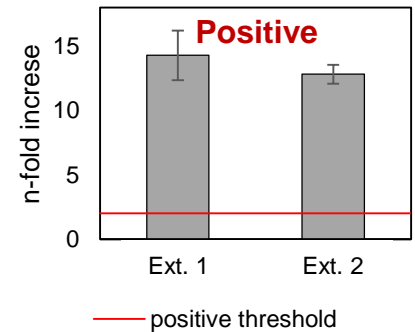
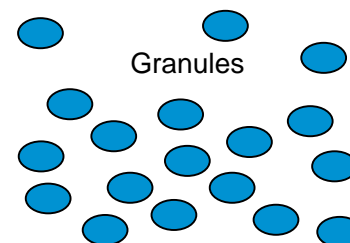
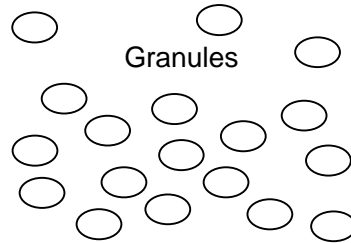
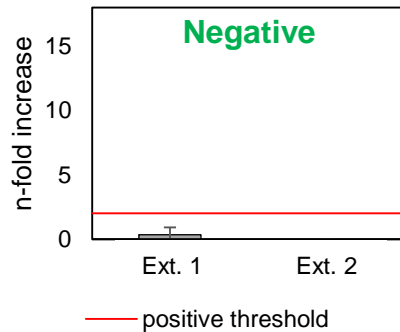
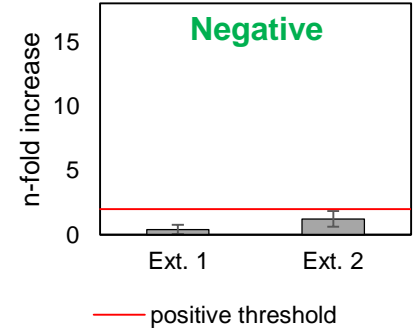
Only **printed AND recycled** materials scored Ames positive!

Unprinted foil

Printed foil

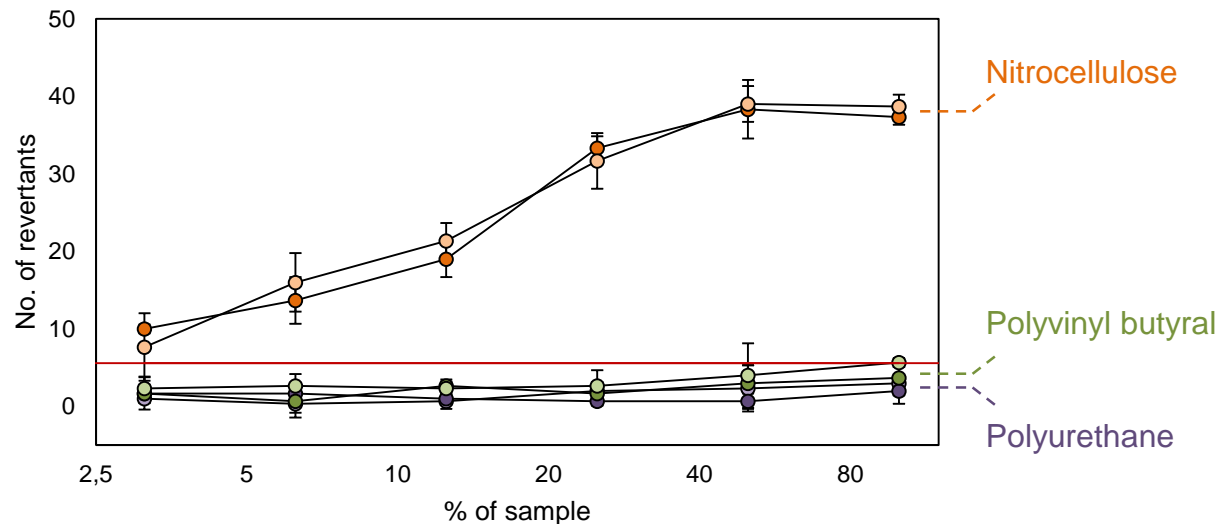
Recycling  
process

Recycling  
process



# Binders of printing inks have influence on results

## rPP from white printed materials



- Nitrocellulose (M1)
- Nitrocellulose (M2)
- Polyurethane (M1)
- Polyurethane (M2)
- Polyvinyl butyral (M1)
- Polyvinyl butyral (M2)
- Positive threshold

rPP recyclates were produced from white printed input streams.

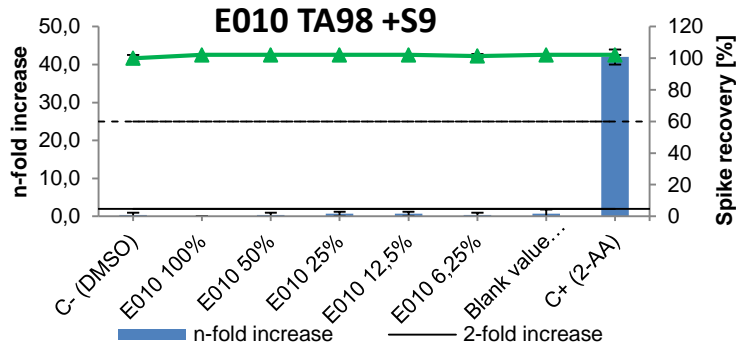
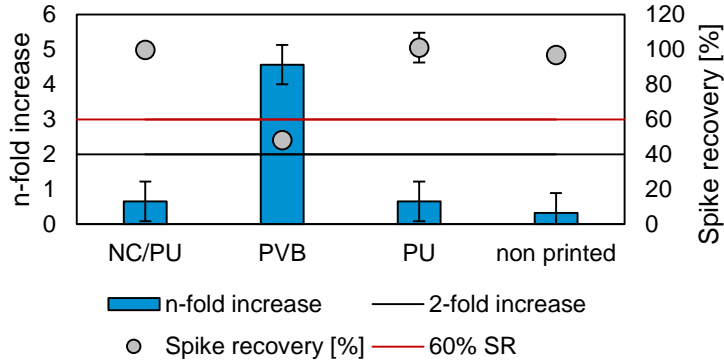
Printing inks based on:

- Nitrocellulose (NC)
- Polyvinyl butyral (PVB)
- Polyurethane (PU)

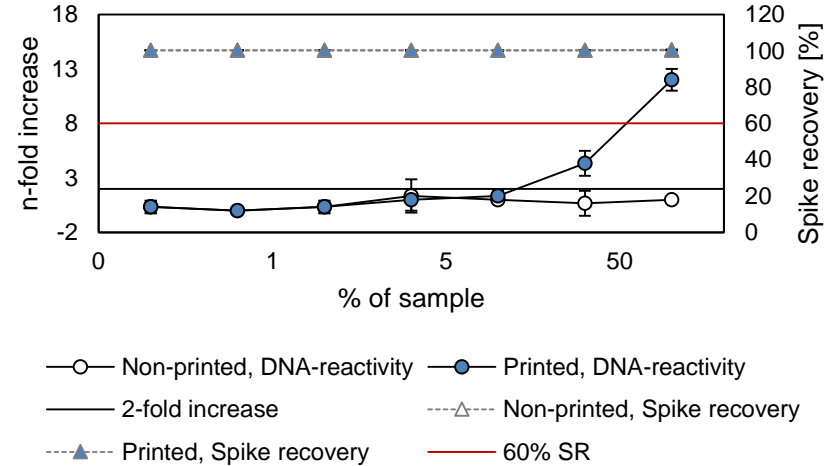
Recyclates from nitrocellulose-based printed samples are strongly Ames positive.

# Unprinted references showed Ames negative results

Blue & white printed foils



Acryalte, multi-coloured



Wish from Migratox Industryboard (Feedback Meeting on Dec. 6<sup>th</sup> 2022):  
**Further investigation of virgin printed samples.**

The majority of printed samples do not show DNA-reactivity!

However: Ames test activity was detected for some samples  
(different binder chemistries!)

## Worst case: Printed side tested directly!

Sample	Type	Colour	Ames (TA98 +S9)	Sample	Type	Colour	Ames (TA98 +S9)
1	-	Multi-colored	-	20	UV-based	Black	-
2	-	Multi-colored	-	21	UV-based	Black	-
3	-	Multi-colored	-	22	UV-based	Blue (Cyan)	-
4	-	Multi-colored	+	23	UV-based	Blue (Cyan)	-
5	-	Multi-colored	+	24	UV-based	Red (Magenta)	-
6	Acrylate	Multi-colored	+	25	UV-based	Red (Magenta)	-
7	Acrylate	Pink	-	26	UV-based	Yellow	-
8	Acrylate	Purple	-	27	UV-based	Yellow	-
9	Acrylate	Yellow	-	28	Water-based	Black	-
10	NC	Blue	-	29	Water-based	Black	-
11	NC	Multi-colored	+	30	Water-based	Blue (Cyan)	+
12	NC	N.a.	-	31	Water-based	Blue (Process blue)	+
13	NC	Orange	-	32	Water-based	Red (Magenta)	-
14	NC	White	-	33	Water-based	Red (Rubine red)	+
15	NC/PU	White	-	34	Water-based	Reflex Blue	+
16	NC/PU	White / Blue	-	35	Water-based	White (Transparent)	-
17	PU	Multi-colored	-	36	Water-based	Yellow	-
18	PU	White / Blue	-	37	Water-based	Yellow	-
19	PVB	White / Blue	+	/	/	/	/

The majority of printed samples do not show DNA-reactivity!

However: Ames test activity was detected for some samples  
(different binder chemistries!)

NC-based printing inks are NOT especially critical if not recycled!

**Worst case: Printed side tested directly!**

Sample	Type	Colour	Ames (TA98 +S9)	Sample	Type	Colour	Ames (TA98 +S9)
1	-	Multi-colored	-	20	UV-based	Black	-
2	-	Multi-colored	-	21	UV-based	Black	-
3	-	Multi-colored	-	22	UV-based	Blue (Cyan)	-
4	-	Multi-colored	+	23	UV-based	Blue (Cyan)	-
5	-	Multi-colored	+	24	UV-based	Red (Magenta)	-
6	Acrylate	Multi-colored	+	25	UV-based	Red (Magenta)	-
7	Acrylate	Pink	-	26	UV-based	Yellow	-
8	Acrylate	Purple	-	27	UV-based	Yellow	-
9	Acrylate	Yellow	-	28	Water-based	Black	-
10	NC	Blue	-	29	Water-based	Black	-
11	NC	Multi-colored	+	30	Water-based	Blue (Cyan)	+
12	NC	N.a.	-	31	Water-based	Blue (Process blue)	+
13	NC	Orange	-	32	Water-based	Red (Magenta)	-
14	NC	White	-	33	Water-based	Red (Rubine red)	+
15	NC/PU	White	-	34	Water-based	Reflex Blue	+
16	NC/PU	White / Blue	-	35	Water-based	White (Transparent)	-
17	PU	Multi-colored	-	36	Water-based	Yellow	-
18	PU	White / Blue	-	37	Water-based	Yellow	-
19	PVB	White / Blue	+	/	/	/	/

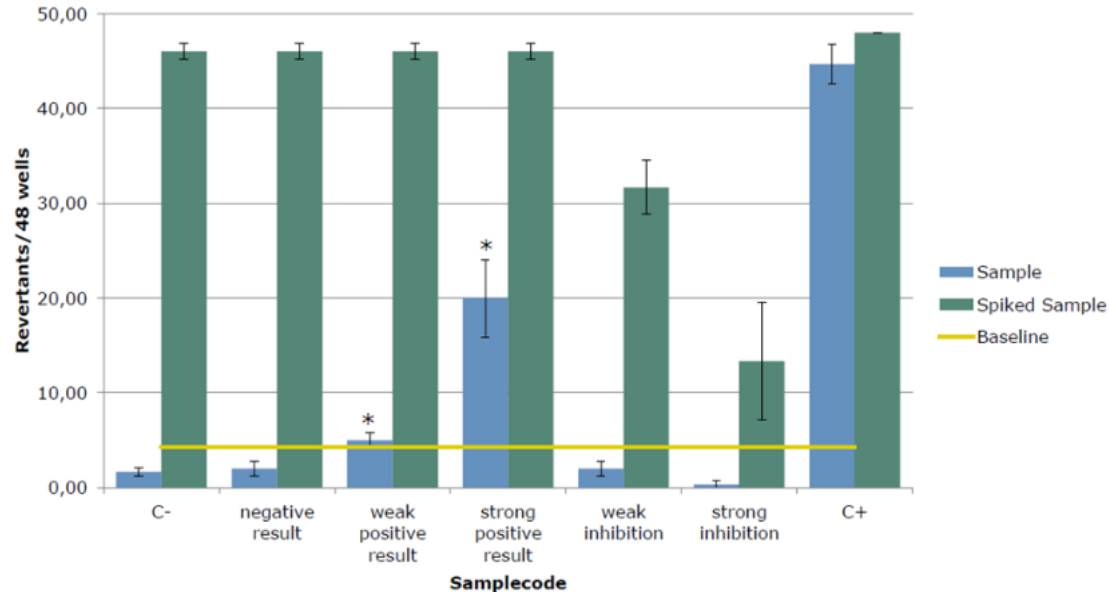


## Material & Methods – Sample preparation\*

- **Extraction conditions:** 95% ethanol, 10 days, 60 °C
- **Extraction:** Total immersion, direct contact with printed outside
- **Concentration:** Evaporation and transfer to DMSO
- **Concentration factor:** ~300
- **Solvent blank:** 95 % ethanol, undergoes all steps without sample

\*Source: Rainer, Mayrhofer et. al. (2019): **Mutagenicity assessment of food contact material migrates with the Ames MPF assay.**  
<https://doi.org/10.1080/19440049.2019.1634841>

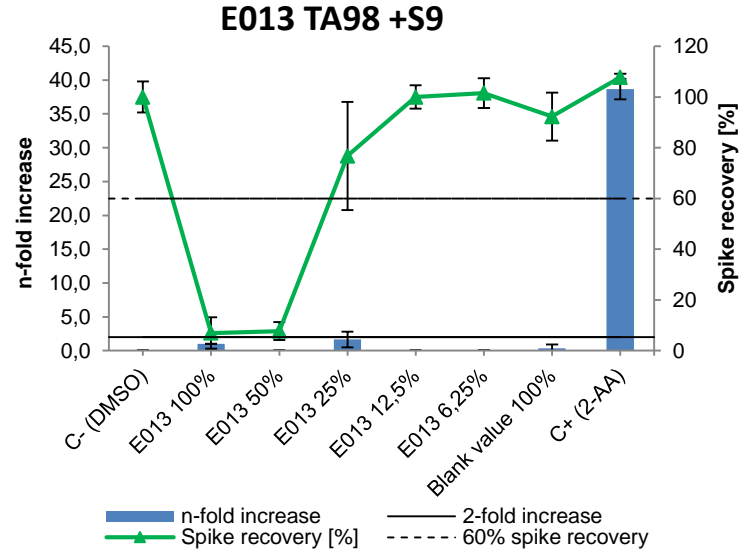
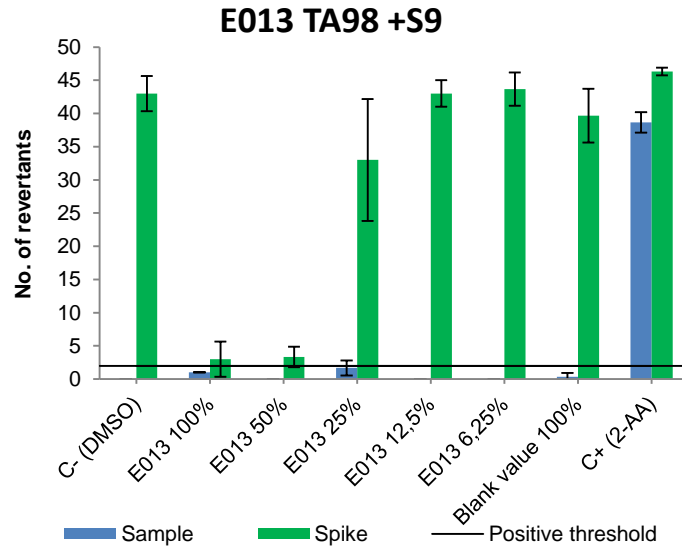
## Ames MPF™ - Evaluation



*Baseline = (Mean + standard deviation of the negative control)*

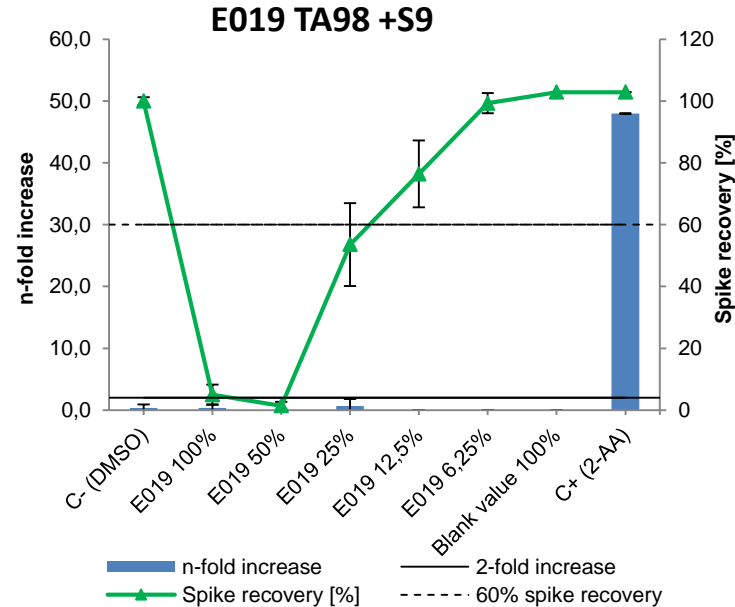
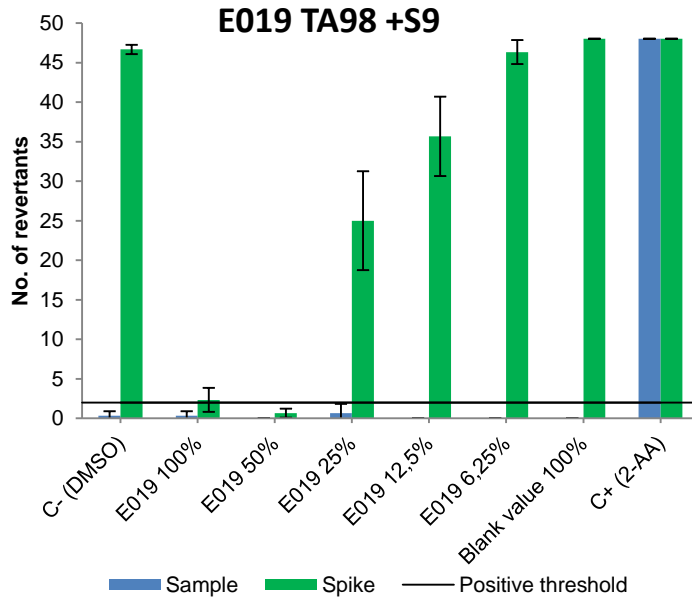
- **Positive result:** Mean number of revertants surpasses the **positive threshold (baseline multiplied by 2)**
- **Inhibiting result:** Spike recovery is below 60 % compared to the negative control (C-)

## Inhibiting result: Sample E013 (Yellow ink foil, UV cured)



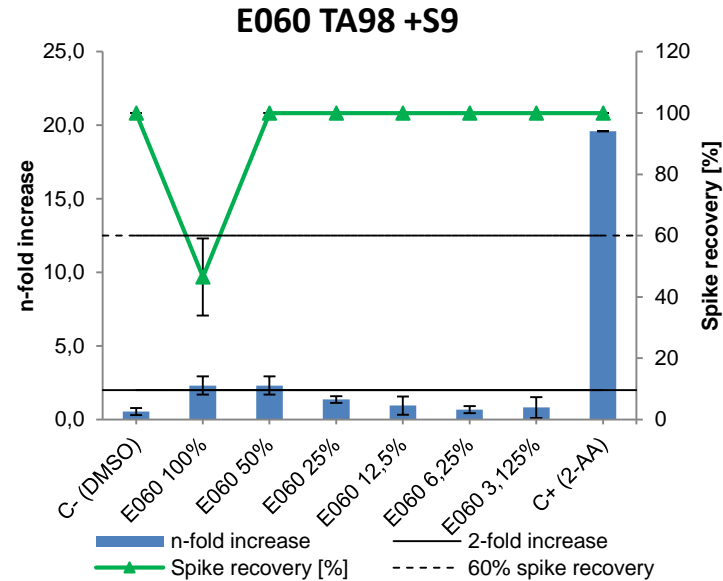
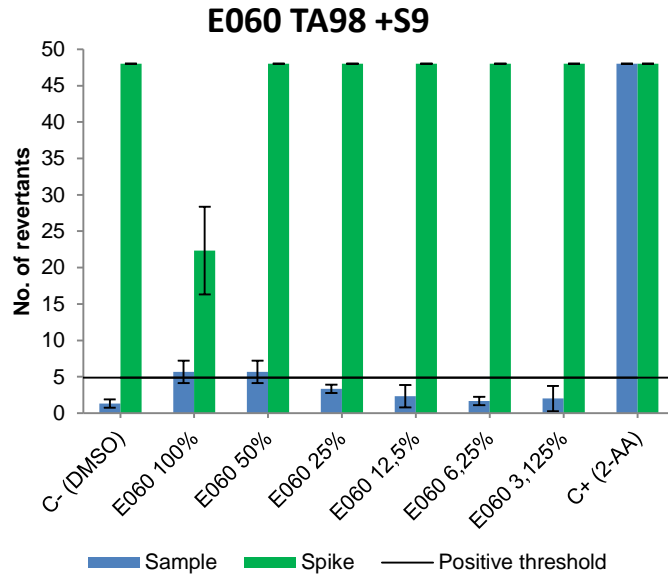
> Sample E013 was inhibiting up to a dilution of 50 % (spike recovery around 7 %). The positive threshold was 2.0

## Inhibiting result: Sample E019 (Black ink foil, UV cured)



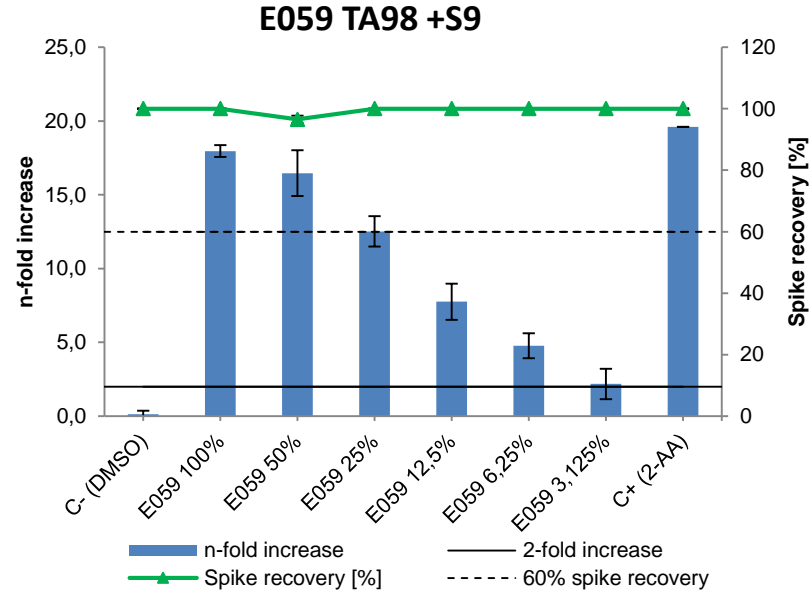
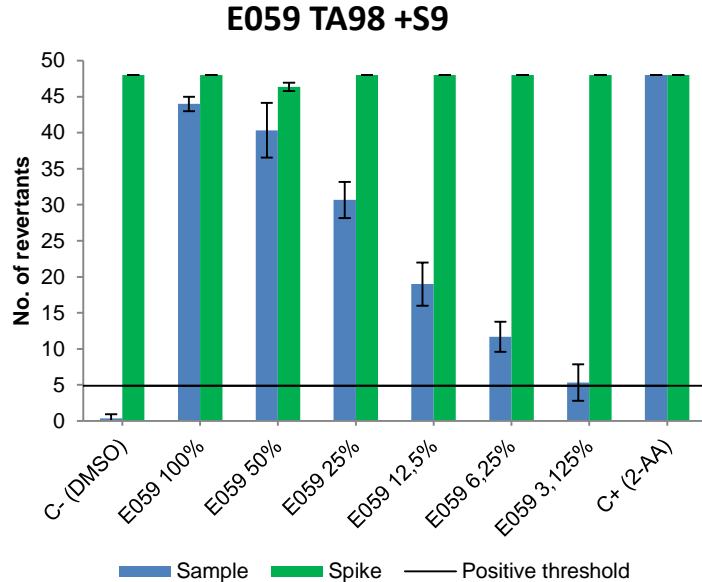
> Sample E019 was inhibiting up to a dilution of 25 % (spike recovery around 5 %). The positive threshold was 2.0

## Positive result: Sample E060 (Red ink, water based)



> Sample E060 was weak positive up to a dilution of 50% (n-fold induction 2.3). The undiluted sample was inhibitory (spike recovery: 46.5 %)

## Positive result: Sample E059 (Water based ink, Reflex Blue )



> Sample E058 was positive up to a dilution of 3.125 %. The positive threshold was 4.9

# Conclusions

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- Most virgin printed samples do not show DNA-reactivity, even if tested at extreme conditions at the printed outside.
- However: DNA-reactivity was detected in some virgin printed samples (direct testing of printed outside)
  - No obvious connection to binders!
  - First indications that certain colour shades\* could be a risk factor.  
\*(Detailed information (material specification) about pigments not available.)
- More information on the formulation of the inks (solvents, binders, pigments,...) would be necessary



Thank you for your attention!

## **Open Questions?**

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