Genotoxic and acute toxic properties of selected synthetic cannabinoids

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EU-Project: SPICE II Plus

Topics

- Background
- Methods
- Aims of the study
- Results
- Conclusions

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Background

The toxicological properties of a varieties of synthetic cannabinoids were evaluated in the frame of first EU-Spice-Project.

In general no dramatic effects were seen in regard to:

1. ***Acute Toxicity***
2. ***Biological properties***
3. ***Immunological effects***

The most intresting observation, was the evidence for genotoxic effects in different human derived cell lines.

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Publications (2013-2014)

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MOLECULAR TOXICOLOGY

Toxicological profiles of selected synthetic cannabinoids showing high binding affinities to the cannabinoid receptor subtype CB_1

Verena J. Koller · Gerhard J. Zlabinger · Volker Auwärter · Sabine Fuchs · Siegfried Knasmueller

Toxicology and Applied Pharmacology 277 (2014) 164–171

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Investigation of the in vitro toxicological properties of the synthetic cannabimimetic drug CP-47,497-C8

Verena J. Koller [a], Volker Auwärter [b], Tamara Grummt [c], Bjoern Moosmann [b], Miroslav Mišík [a], Siegfried Knasmüller [a,*]

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Aim of the Study I

Since genotoxic effects have no or very low trashhold levels. It can not excluded that synthetic cannabinoids may cause damage in users, in particular in epithelial cells of the respiratory tract.

DNA-damage leads to adverse health effects

- **In somatic cells: cancer, aging**
- **In germ cells: infertility, malformations**

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Aim of the Study II

We tested six cannabinoids in three different test systems with human lymphocytes

1. **Single Cell Gel Electrophoresis Assay (SCGE assay)**
2. **Micronucleus assay (MN assay)**
3. ***Salmonella*microsome assay (Ames Test)**

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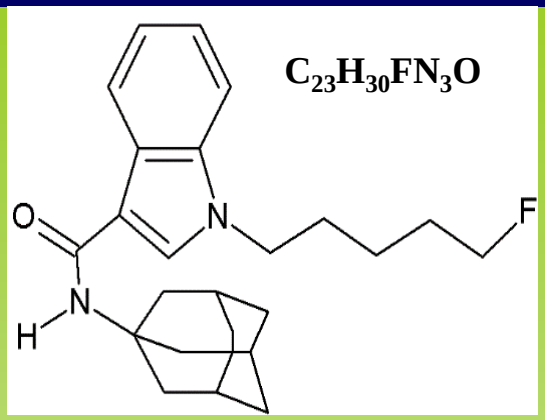
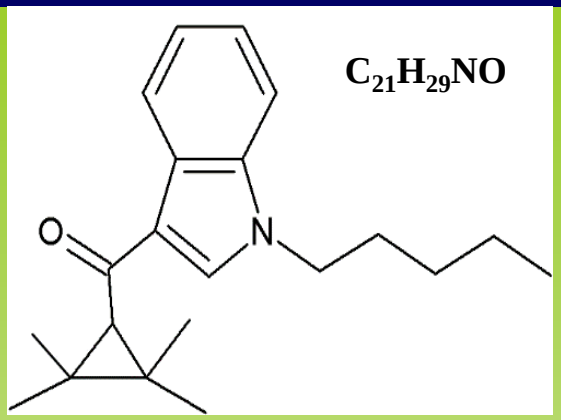
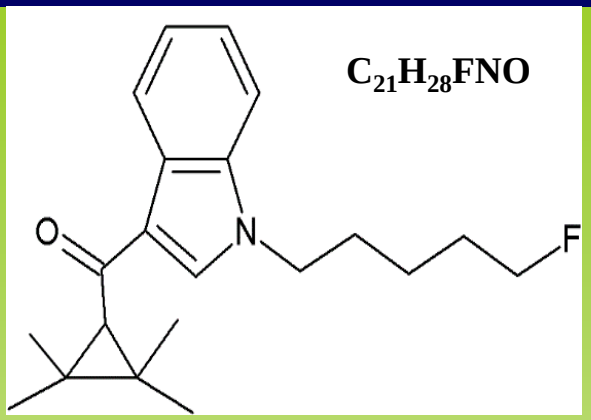
Synthetic cannabinoids



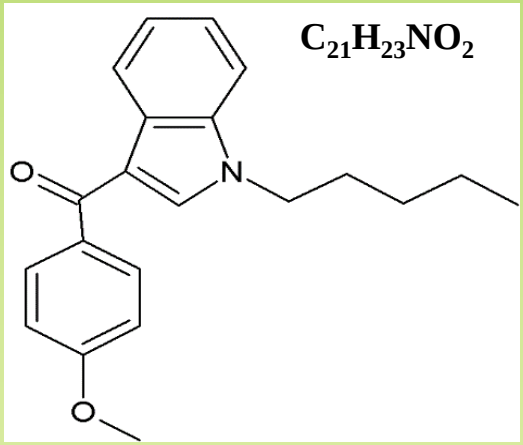
AKB48 N-(5-fluoropentyl) analog



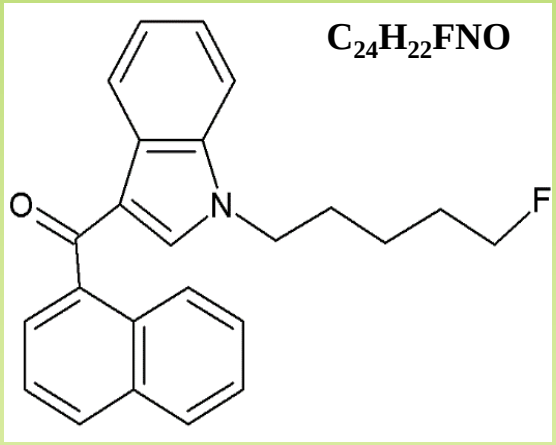
UR-144



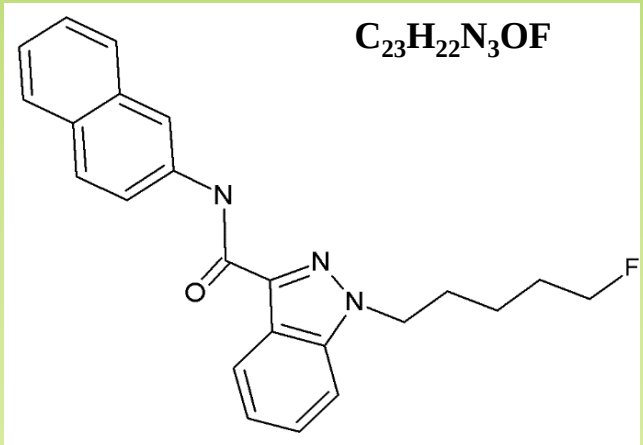
XLR-11



RCS-4



AM-2201



AM-2201 Indazole Carboxamide

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Test compounds

AKB-48-5F is a representative of the adamantoylindoles. It belongs to the „ third generation“ of SC, which appeared on the market in 2012. The structure is very unique in that it contains an amide adjacent to adamantyl ring and also an indazole group.

UR-144 is a tetramethylcyclopropylindole, which was invented by Abbott Laboratories.

XLR-11 is an halogenated analog of UR-144.

RCS-4 is a non-halogenated benzoylindole with a methoxy substituent on its phenyl ring.

AM-2201 is an aminoalkylindole type of SC. This research chemical was developed by Alexandros Makriyannis in 2007.

AM-2201 indazole carboxamide is a naphthoylindazole substance with a carboxamide moiety.

Test System I
Comet assay with human lymphocytes

The single cell gel elektrophoresis assay (SCGE assay) is based on the measurement of induction of DNA-migration in an electric field. The size and intensity of the comets are indicative for the extent of DNA-damage.

Comet assay can be used for the detection of:

- **single strand breaks**
- **double strand breaks**
- **alkali labile sites**
- **incomplete excision repair sites**
- **DNA crosslinks**
- **oxidized purines and pyrimidines**
- **DNA repair**

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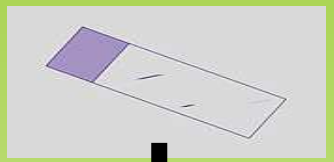
Blood collection
Lymphocyte Isolation

Lymphocytes were incubated with different SC

Washing and centrifugation



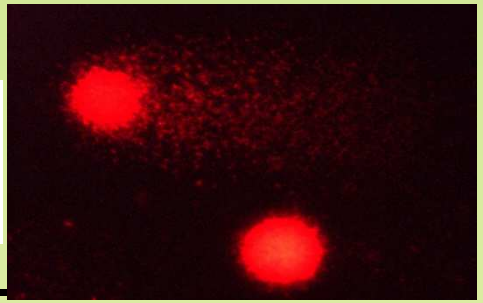
Lysis(1h)

Alkaline unwinding

Elektrophoresis (300 mA, 25 V)

DNA-staining and analysing

(Ethidiumbromide)



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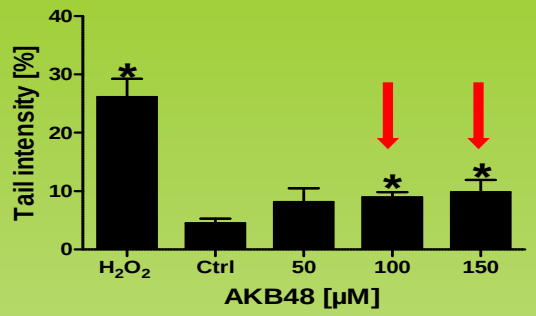
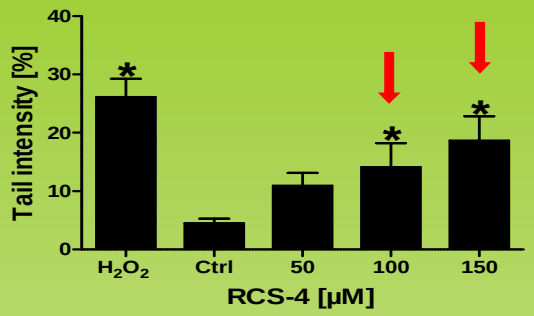
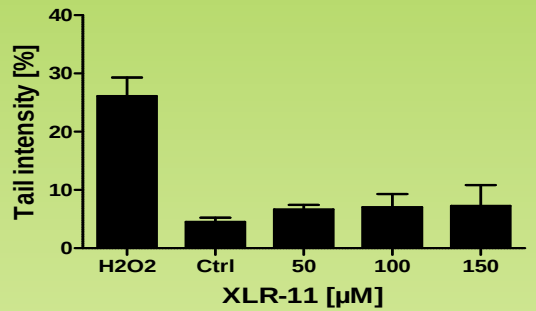
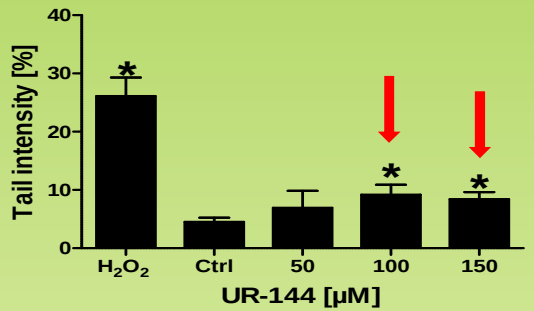
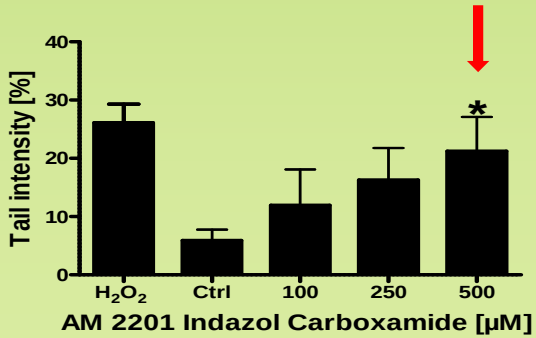
Results of the comet assay

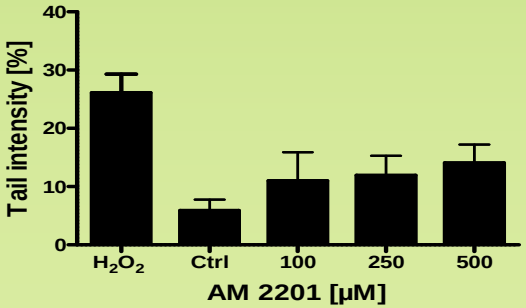












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Summary of the results of the Comet assay

Significant DNA-damage was seen with AKB-48, RCS-4 and UR-144 at concentrations between 100 and 160 µM.

Furthermore, a significant effect was seen with AM-2201-IC with a concentration of 500 µM.

Since „ Comets“ disapear as a consequence of DNA-repair it is difficult to draw conclusions about the toxicological consequences.

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The next series of experiments was performed to find out if the COMETS cause persistence DNA-damage at the „ Chromosomal“ level. We know that chromosomal aberrations cause adverse health effects in humans.

Increased levels of micronuclei in peripheral lymphocytes are correlated with increased cancer risks in humans. (*Bonassi et al., Mutagenesis 2011*)

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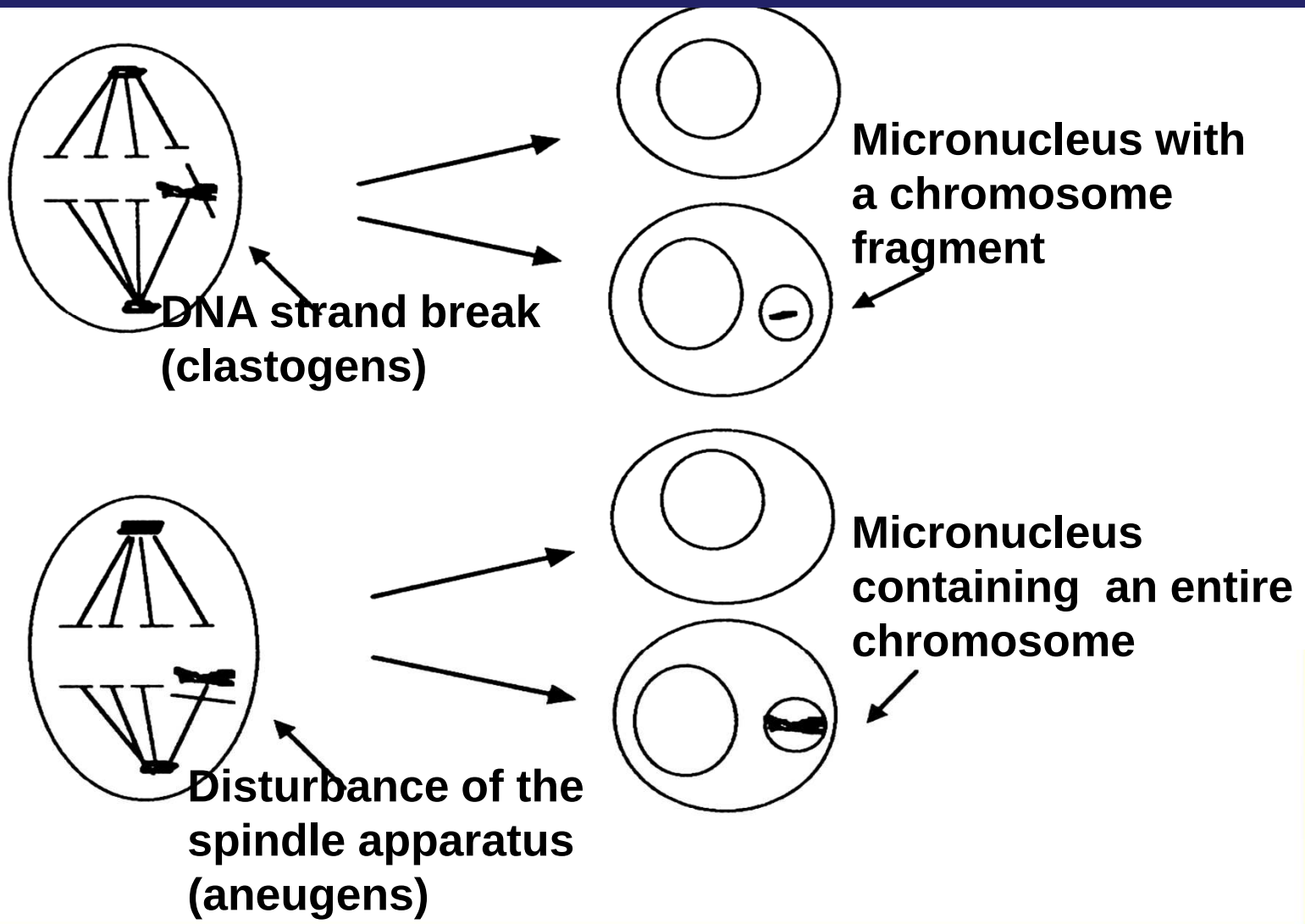
Test System II
Micronucleus assay (MN) with human lymphocytes

MNi are indicative for structural and numerical chromosomal aberrations. They were monitored with the cytokinesis-block micronucleus cytome assay (CBMN) which is based on use of cytocalasin B.

MN are formed as a consequence of chromosomal breaks.

In addition also other nuclear anomalies were monitored which are indicative for genetic alterations such as nuclear bridges and nuclear buds.

MN-formation



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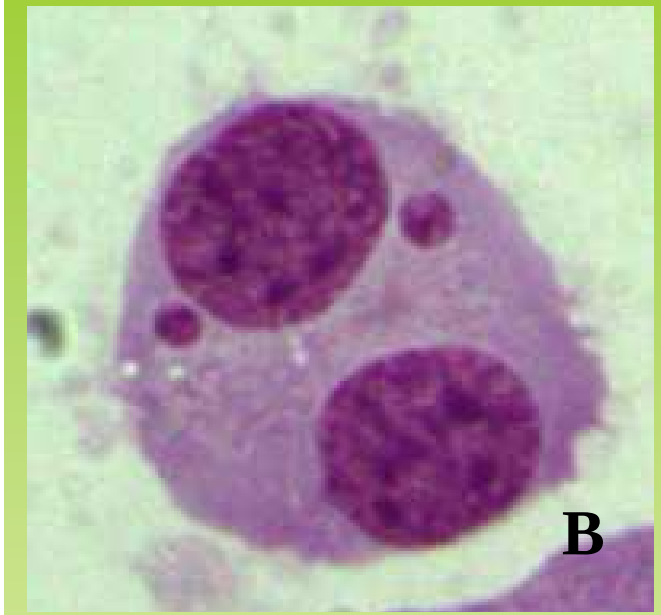
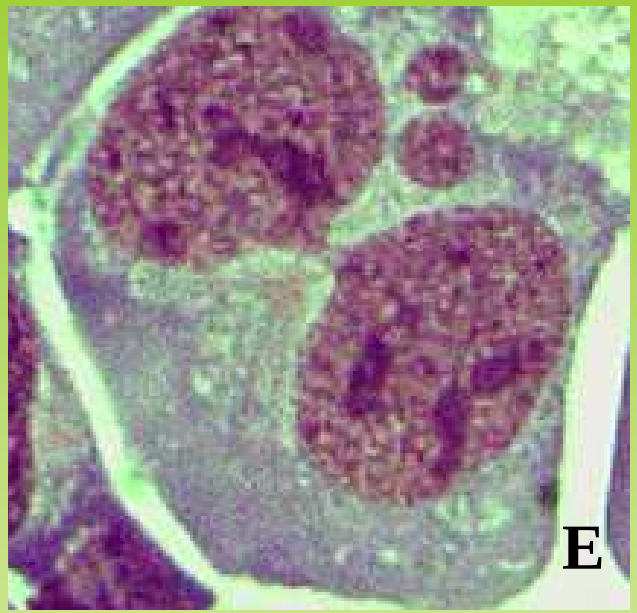
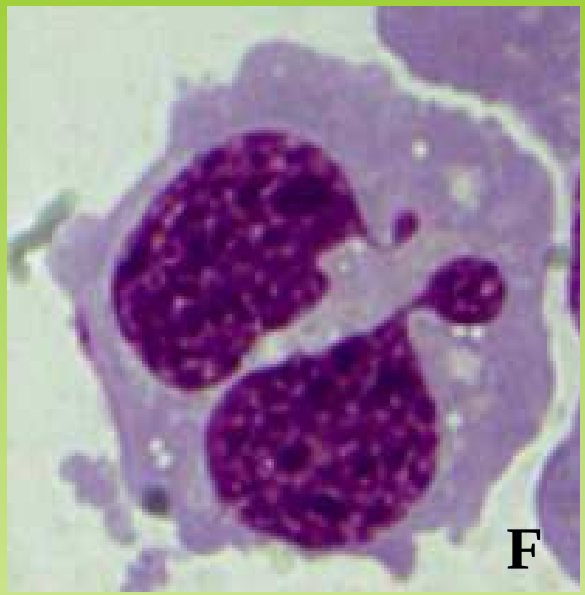
MN-formation in lymphocytes



Binuclated Micronuclei



Nucleoplasmatic bridge



Nuclear buds

B: Binucleated cells
E: Nuclear bridges are dicentric chromosoms
F: Nuclear buds reflect gene amplification

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Results of the MN experiments

		NDI and CT [%]		BN-MN	MN	Nbuds	NPBs
Compound	**Concentration**	**Mean (NDI) ± SD**	**CT [%]**	**Mean [‰] ± SD**	**Mean [‰] ± SD**	**Mean [‰] ± SD**	**Mean [‰] ± SD**
Pos. Ctrl	1 µg/ml	1.739 ± 0.064	26.1	48.360 ± 9.374	50.620 ± 10.100	12.440 ± 5.918	2.658 ± 1.169
Neg. Ctrl	0 µM	2.028 ± 0.144	-2.8	4.143 ± 0.524	4.257 ± 0.496	2.961 ± 1.555	1.674 ± 0.755
5F-AKB-48	25 µM	1.906 ± 0.155	9.4	5.462 ± 0.816	5.793 ± 0.973	2.688 ± 0.862	1.781 ± 0.450
	50 µM	1.717 ± 0.128	28.3	7.800 ± 1.160*	8.024 ± 1.374*	4.348 ± 1.092	2.374 ± 0.725
	75 µM	1.598 ± 0.109	40.2*	8.431 ± 1.611*	8.546 ± 1.707*	4.833 ± 1.138	2.482 ± 0.672
	100 µM	1.551 ± 0.051	44.9*	9.757 ± 1.044*	10.090 ± 1.540*	4.346 ± 1.521	3.226 ± 1.369*
	150 µM	1,054 ± 0.022	**94.6***	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.
XLR-11	25 µM	1.855 ± 0.195	14.5	7.025 ± 1.755	7.258 ± 1.864	4.312 ± 1.947	1.814 ± 0.966
	50 µM	1.690 ± 0.215	31.0	6.337 ± 2.502	6.458 ± 2.700	3.130 ± 0.769	2.446 ± 0.876
	75 µM	1.586 ± 0.143	41.4*	8.536 ± 1.320	8.783 ± 1.016	4.475 ± 1.023	2.770 ± 0.650
	100 µM	1.416 ± 0.132	58.4*	14.510 ± 4.395*	16.000 ± 5.708*	9.081 ± 3.035*	6.466 ± 3.024*
	150 µM	1.074 ± 0.049	**92.6***	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.
UR-144	25 µM	1.777 ± 0.146	22.3	5.235 ± 1.629	5.739 ± 1.941	2.958 ± 0.937	1.865 ± 0.871
	50 µM	1.429 ± 0.167	57.1*	7.665 ± 0.688	8.303 ± 1.433	4.540 ± 2.218	2.560 ± 2.805
	75 µM	1.241 ± 0.075	**75.9***	7.199 ± 0.626	7.199 ± 0.626	2.573 ± 2.095	2.443 ± 0.367
	100 µM	1.181 ± 0.083	**81.9***	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.
	150 µM	1.142 ± 0.135	**85.8***	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.

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Results of the MN experiments

		NDI and CT [%]		BN-MN	MN	Nbuds	NPBs
Compound	**Concentration**	**Mean (NDI) ± SD**	**CT [%]**	**Mean [‰] ± SD**	**Mean [‰] ± SD**	**Mean [‰] ± SD**	**Mean [‰] ± SD**
Pos. Ctrl	1 µg/ml	1.739 ± 0.064	26.1	48.360 ± 9.374	50.620 ± 10.100	12.440 ± 5.918	2.658 ± 1.169
Neg. Ctrl	0 µM	2.028 ± 0.144	-2.8	4.143 ± 0.524	4.257 ± 0.496	2.961 ± 1.555	1.674 ± 0.755
RCS-4	25 µM	1.785 ± 0.154	21.5	6.452 ± 1.237	6.923 ± 0.963	3.734 ± 1.355	2.720 ± 1.236
	50 µM	1.518 ± 0.215	48.2	n.e. ± n.e.	n.e. ± n.e.	n.e. ± n.e.	n.e. ± n.e.
	75 µM	1.250 ± 0.206	**75.0***	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.
	100 µM	1.189 ± 0.170	**81.1***	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.
	150 µM	1.071 ± 0.112	**92.9***	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.
AM 2201 IC	25 µM	1.689 ± 0.114	31.1	5.520 ± 0.728	5.640 ± 0.840	2.592 ± 0.901	1.800 ± 1.066
	50 µM	1.530 ± 0.114	47.0*	6.293 ± 1.680	6.823 ± 1.851	2.930 ± 0.526	2.017 ± 0.982
	75 µM	1.391 ± 0.141	60.9*	9.713 ± 3.945*	9.713 ± 3.945*	4.879 ± 1.909	4.483 ± 2.097*
	100 µM	1.285 ± 0.148	**71.5***	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.
	150 µM	1.243 ± 0.180	**75.7***	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.
AM 2201	25 µM	1.714 ± 0.118	28.6	5.026 ± 1.168	5.422 ± 1.270	2.387 ± 0.779	1.679 ± 0.731
	50 µM	1.530 ± 0.143	47.0*	7.236 ± 1.422	7.755 ± 1.521	3.207 ± 1.540	2.337 ± 1.115
	75 µM	1.436 ± 0.185	56.4*	8.147 ± 1.535	8.966 ± 2.394	3.447 ± 1.239	3.109 ± 0.725
	100 µM	1.332 ± 0.208	**66.8***	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.
	150 µM	1.233 ± 0.115	**76.7***	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.	n.d. ± n.d.

Summary of the results of the MN assay

AKB-48-5F, XLR-11 and AM-2201-IC induced MN-formation in lymphocytes at concentrations between 50-100 µM.

DNA-breaks which lead to comet-formation are only partly repaired and cause damage at the CHROMOSOMAL LEVEL

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Test System III
Salmonella /Microsome assay
Ames assay

The most widely used test for routine screening of the genotoxicity of chemicals. If positive results are obtained with a chemical, further tests for should be performed.

Current data base ≥ 15.000 chemicals

We used two strains, TA98 and TA100 with and without S9-enzyme mix which mimik the activation of different compounds by liver enzymes.

TA98 detects frame-shift mutations, TA100 gene mutations.

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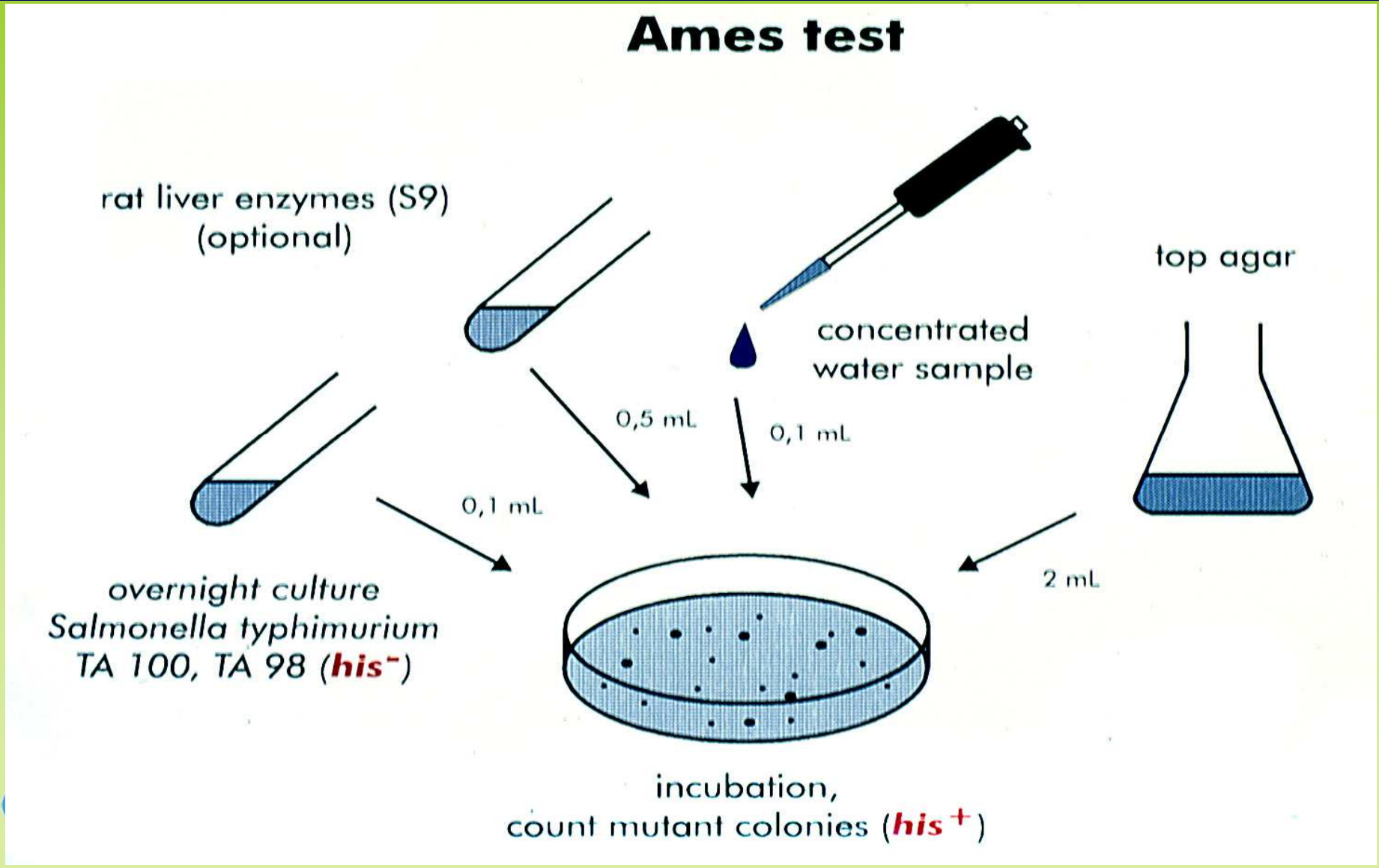
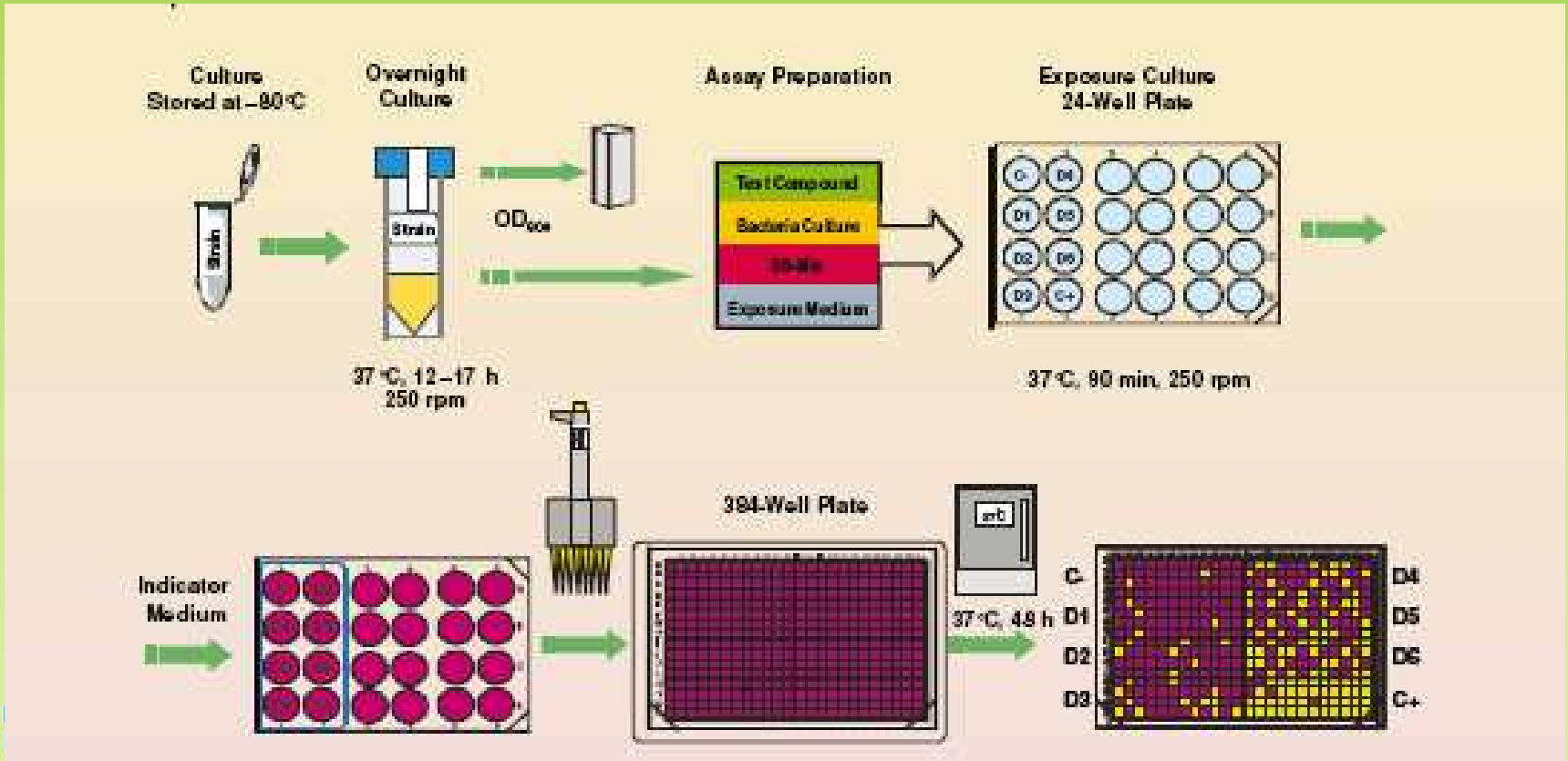
Classical Ames assay



Ames microplate formate assay
Ames MPF™ (Xenometrix)

Mutagenicity testing with the liquid microplate format was performed according to OECD Guideline 471 with strain TA98 and TA100 strains.



Results of the Ames assay

Test series I		TA98-S9		TA98-S9		TA100-S9		TA100+S9	
Compound	**[mM]**	**Mean ± SD**	**FI**	**Mean ± SD**	**FI**	**Mean ± SD**	**FI**	**Mean ± SD**	**FI**
Pos. Ctrl.		**28.3 ± 1.2`***	**22.8***	**48.0 ± 0.0***	**19.3***	**48.0 ± 0.0***	**11.3***	**36.0 ± 1.0***	**6.9***
Neg. Ctrl.		0.7 ± 0.6	0.5	1.3 ± 1.2	0.5	3.7 ± 0.6	0.9	3.7 ± 1.5	0.7
AMIC	1.00	0.0 ± 0.0	0.0	0.7 ± 0.6	0.3	3.7 ± 1.2	0.9	5.7 ± 2.3	1.1
	0.10	0.3 ± 0.6	0.3	1.3 ± 1.2	0.5	3.0 ± 1.0	0.7	5.7 ± 2.9	1.1
	0.01	0.7 ± 0.6	0.5	1.0 ± 1.7	0.4	3.3 ± 0.6	0.8	5.7 ± 1.5	1.1
AM2201	1.00	0.0 ± 0.0	0.0	0.3 ± 0.6	0.1	1.7 ± 0.6	0.4	4.3 ± 1.2	0.8
	0.10	0.7 ± 0.6	0.5	1.0 ± 1.0	0.4	4.7 ± 2.1	1.1	4.3 ± 2.3	0.8
	0.01	0.3 ± 0.6	0.3	0.0 ± 0.0	0.0	3.3 ± 0.6	0.8	3.3 ± 1.5	0.6
RCS-4	1.00	1.0 ± 1.7	0.8	1.7 ± 0.6	0.7	2.7 ± 0.6	0.6	**8.0 ± 1.7***	1.5
	0.10	0.3 ± 0.6	0.3	3.0 ± 1.7	1.2	4.7 ± 0.6	1.1	4.0 ± 2.6	0.8
	0.01	0.3 ± 0.6	0.3	0.7 ± 1.2	0.3	3.7 ± 1.2	0.9	4.0 ± 3.5	0.8

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Results of the Ames assay

Test series II		TA98-S9		TA98-S9		TA100-S9		TA100+S9	
Compound [mM]		**Mean ± SD**	**FI**	**Mean ± SD**	**FI**	**Mean ± SD**	**FI**	**Mean ± SD**	**FI**
Pos. Ctrl.		**41.7 ± 1.5***	**45.7***	**48.0 ± 0.0***	**19.3***	**48.0 ± 0.0***	**5.3***	**34.7 ± 0.6***	**4.4***
Neg. Ctrl.		0.3 ± 0.6	0.4	1.3 ± 1.2	0.5	8.0 ± 1.0	0.9	7.3 ± 0.6	0.9
AKB48	1.00	0.7 ± 1.2	0.7	2.0 ± 1.0	0.8	6.3 ± 2.9	0.7	9.3 ± 5.1	1.2
	0.10	1.3 ± 1.5	1.5	0.3 ± 0.6	0.1	7.7 ± 2.1	0.9	6.0 ± 2.0	0.8
	0.01	1.0 ± 1.0	1.1	0.7 ± 0.6	0.3	6.3 ± 3.1	0.7	5.7 ± 2.3	0.7
UR-144	1.00	1.0 ± 0.0	1.1	0.3 ± 0.6	0.1	5.3 ± 3.2	0.6	6.3 ± 4.2	0.8
	0.10	1.7 ± 1.2	1.8	0.7 ± 1.2	0.3	7.7 ± 3.2	0.9	6.3 ± 4.2	0.8
	0.01	0.7 ± 1.2	0.7	1.7 ± 2.1	0.7	3.3 ± 1.2	0.4	7.3 ± 3.2	0.9
XLR-11	1.00	1.0 ± 1.0	1.1	0.7 ± 1.2	0.3	8.3 ± 1.5	0.9	6.0 ± 2.0	0.8
	0.10	0.3 ± 0.6	0.4	1.7 ± 1.2	0.7	10.3 ± 2.1	1.1	6.7 ± 1.2	0.8
	0.01	0.3 ± 0.6	0.4	2.3 ± 1.5	0.9	9.3 ± 2.3	1.0	6.3 ± 2.9	0.8

Summary of Results of the Ames assay

RCS-4 is the only SC which caused a marginal genotoxic effect with TA100 in presence of metabolic activation mix at highest concentration (1mM).

None of the comounds caused posetive results in gene mutation assay with *Salmonella* strains.

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Conclusions

Four out of six SC, namely AKB-48, RCS-4, UR-144 and AM2201 IC which we investigated caused DNA-formation in human lymphocytes.

Three out of six SC caused positive results in MN assay (AKB-48, XLR-11 and AM2201 IC).

These findings indicate that the latter drugs may cause cancer in humans.

The doses which caused MN-formation in lymphocytes are substantially higher than in plasma. However, exposure to higher doses can be expected in the cells of the respiratory tract.

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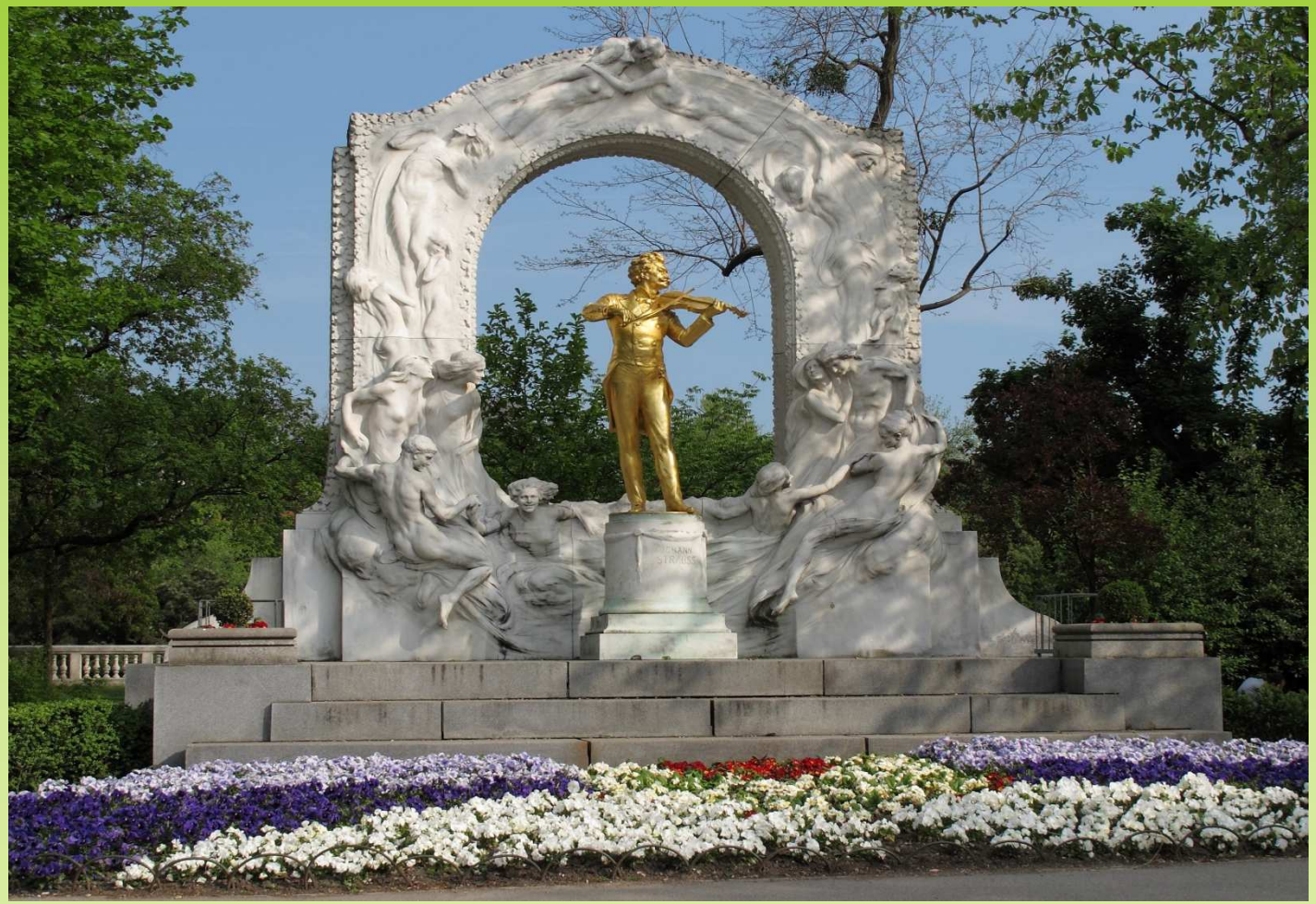
Thank you for your attention



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