

SAFETY TESTING OF ADULT NOVELTIES USING METHODS IN VITRO

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Introduction



- ❑ Adult novelties (sex toys) appeared in ancient times and are widely used particularly in last decades
- ❑ Widespread and prolonged contact with tissues (e. g. Kegel balls recommended for strengthening of pelvic floor muscles - 8 hours/day)
- ❑ Not regularly tested or legally regulated (products may contain hazardous substances e.g. plasticizers, which may have potential biological effects on human health)





Introduction



❑ marketed either as consumer products or medical devices

❑ according to :



General product safety
directive **2001/95/EC**
(Act. 102/2001)

Council directive **93/42/EEC**
On medical devices
(Act. 268/2014)





Objectives of study

- ❑ 20 samples, Czech market
- ❑ Biological tests in vitro
- ❑ ISO standards, OECD test guidelines, standard protocol

Cytotoxicity

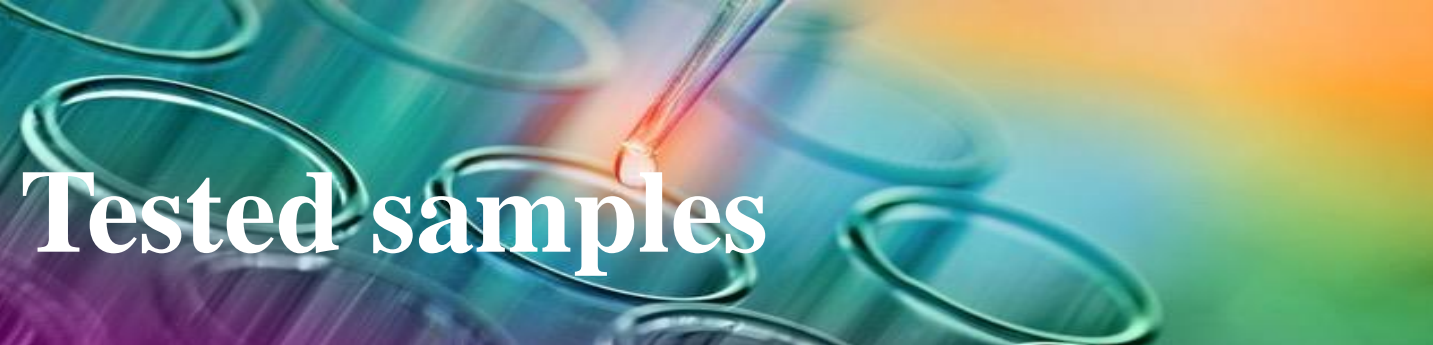
- ISO EN 10993-5: 2009
- 3T3 NRU

Sensitization

- DPRA (OECD TG 442C)
- LuSens (OECD TG 442D)

Endocrine disruption

- YES/ YAS (Xenometrix)



Tested samples

	Product	Material	Country of origin
1	Kegel balls blue	100% silicone	China
2	Kegel balls violet 1	100% silicone	China
3	Kegel balls red 1	100% silicone	China
4	Kegel balls purple	100% silicone	China
5	Vibrator violet 1	100% silicone, ABS	China
6	Twinhead purple	100% silicone	China
7	Artificial vagina	Thermoplastic rubber	China
8	Vibrator violet 2	Styrene-based elastomer	China
9	Kegel balls red 2	ABS	China
10	Kegel balls violet 2	Polyester terephthalate, ABS	China



Tested samples

	Product	Material	Country of origin
11	Strap-on penis sleeve	polyisopren/ latex	Poland
12	Knobbed dildo	PVC	Germany
13	Veined penis	100% latex	Poland
14	Transculent dildo	PVC (Jelly)	China
15	Pleasure balls	silicone	China
16	Dildo violet	PVC (Jelly)	China
17	Love balls pink	ABS	China
18	Power-vibrator	PVC	China
19	Vibratone-balls	PVC	China
20	Orgasm balls green	ABS	China

Samples preparation



❑ 0.2 g/ ml extracted in 5 ml of extractant:

Cytotoxicity

- Culture medium DMEM

DPRA

- Physiological saline solution

LuSens

- DMEM + 1% FBS

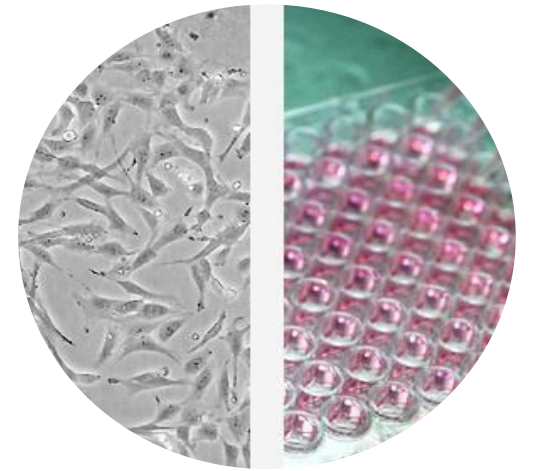
YES/ YAS

- DMSO

❑ Extraction: 24 h, 37 °C, 5% CO₂

❑ Filtration

3T3 NRU cytotoxicity test



- ❑ Balb/c 3T3 mouse fibroblasts
- ❑ 96 well plates, exposed for 24 hours
- ❑ Neutral red uptake relevant to the viability of cells measured on fluorescent reader (FLX800TBI, Biotek)
- ❑ Evaluation:

**NON
CYTOTOXIC**

- 70 – 100%
VIABILITY

**MILD
CYTOTOXICITY**

- 50 – 70%
VIABILITY

**MODERATE
CYTOTOXICITY**

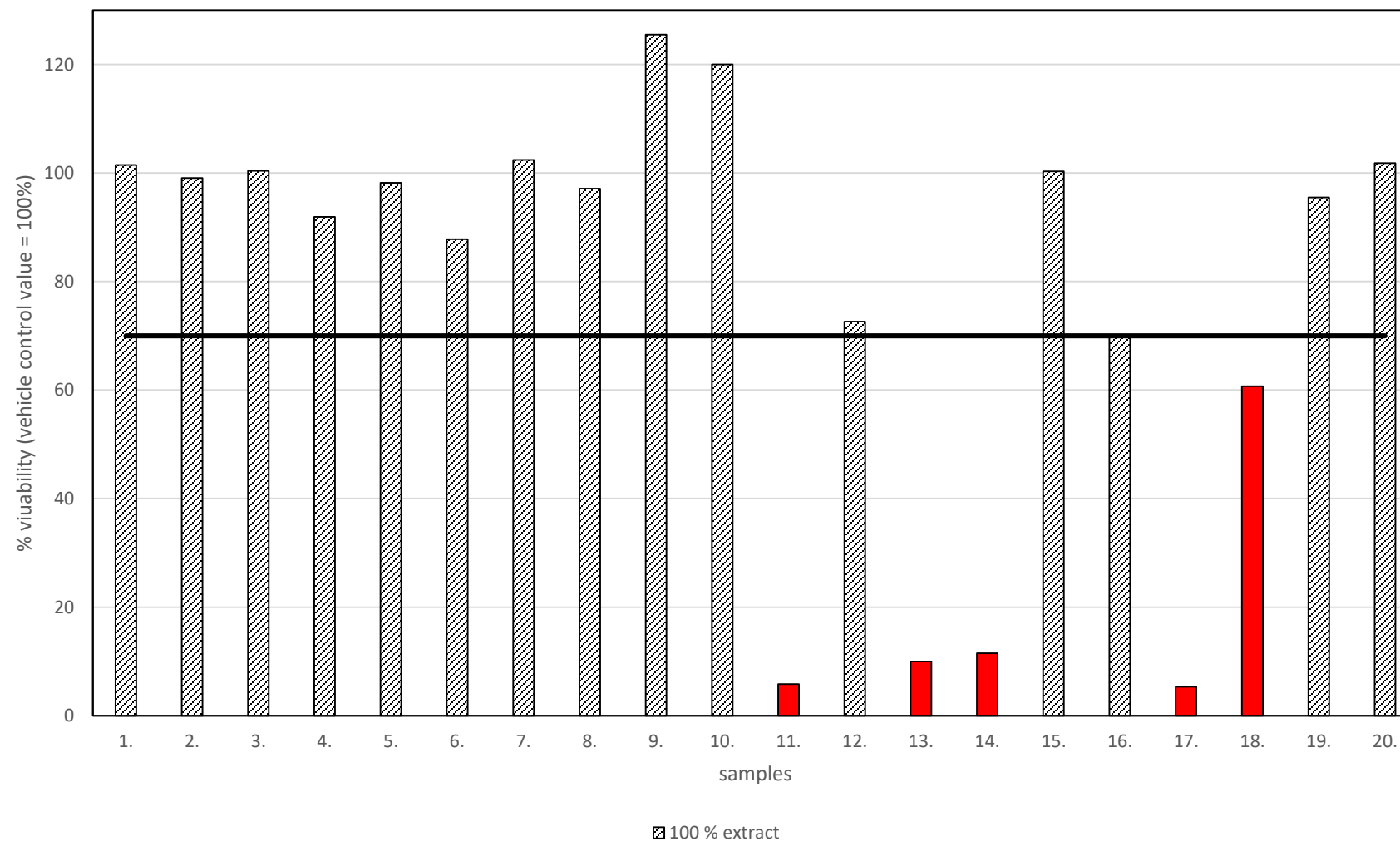
- 30 – 50%
VIABILITY

**SEVERE
CYTOTOXICITY**

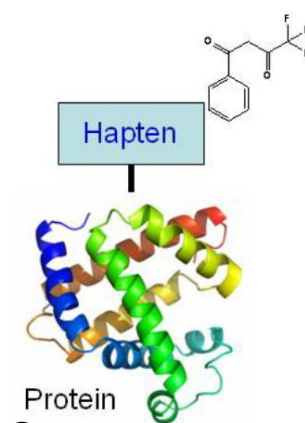
- 0 – 30%
VIABILITY

3T3 NRU cytotoxicity test

Cytotoxic effect in 100% extract:
Samples 11,13,14,17 (severe),
18 (mild)



DPRA



- ❑ In chemico procedure proposed to address the molecular initiating event, protein reactivity, quantifying reactivity of extracted samples towards model synthetic peptide containing cysteine
- ❑ Peptide depletion values (in percentage) are calculated for the prediction model, one of four classes of reactivity
- ❑ 24 hours, HPLC UV detection 220 nm, volume 50 and 250 μ l
- ❑ Evaluation:

MINIMAL REACTIVITY

- <13.8 %

LOW REACTIVITY

- 13.9 – 23.6 %

MEDIUM REACTIVITY

- 23.7 – 98.5 %

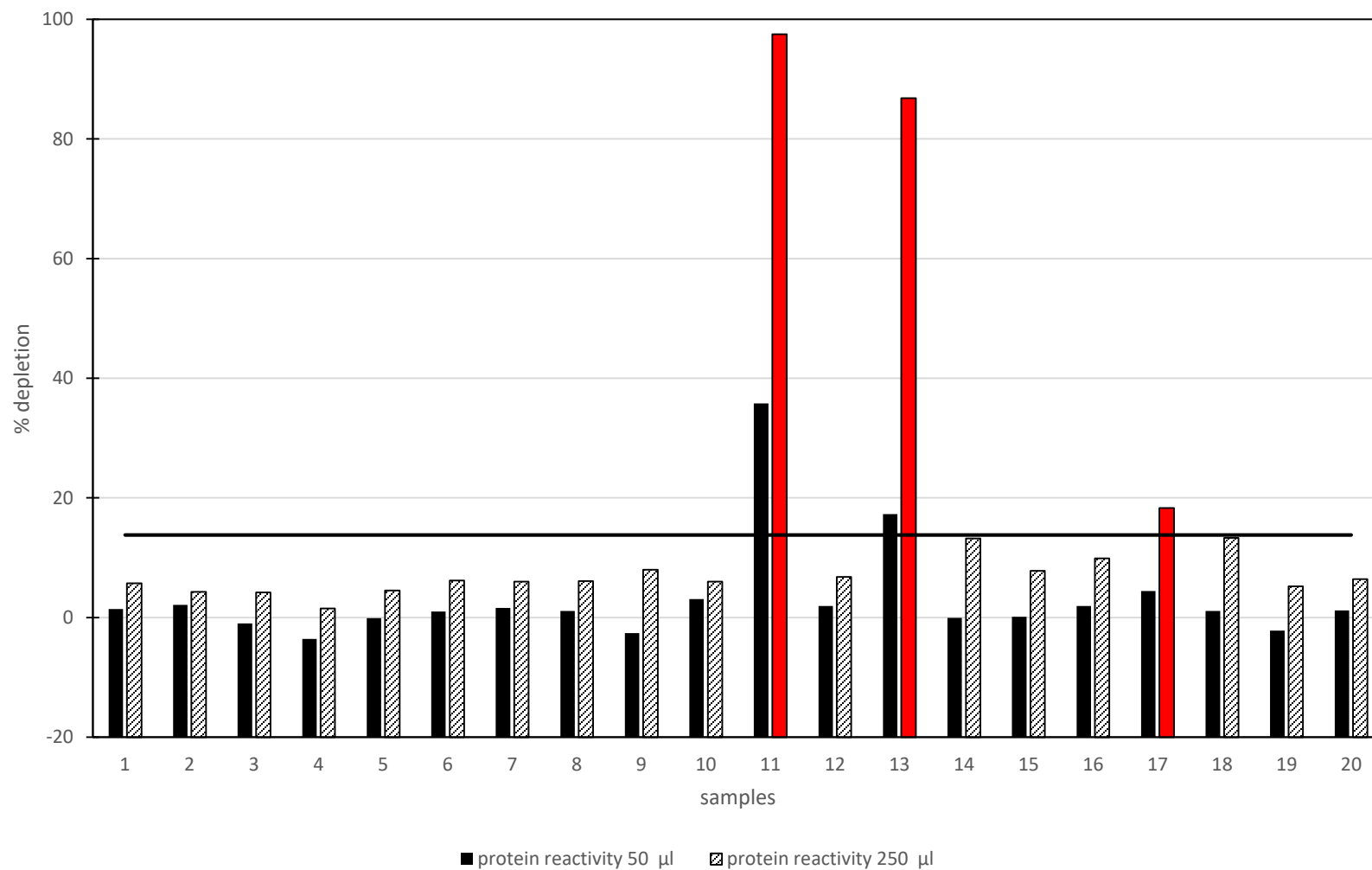
HIGH REACTIVITY

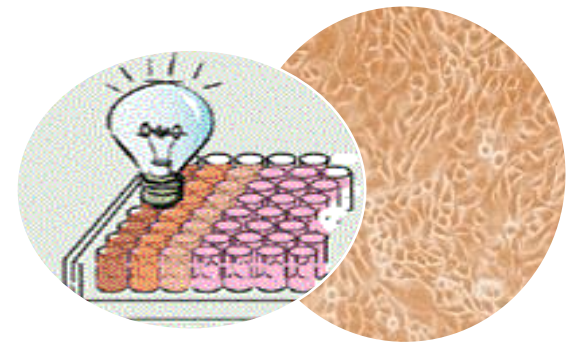
- 98.5 – 100 %



DPRA

Sensitizing potential in 100% extract:
Samples 11,13 (high), 17 (low)





- ❑ Performed on human keratinocytes, genetically modified cells containing luciferase gene under transcription control of promoter and ARE element (up-regulated by skin sensitizers)
- ❑ Signal reflects activation by sensitizer and quantitative measurement of:

luciferase induction
on luminiscence reader (Glo-max)

viability by MTT assay
on spectrophotometer (Eon)

- ❑ 96 well plates, exposed for 48 hours

- ❑ Evaluation:

**SENSITIZING
POTENTIAL**

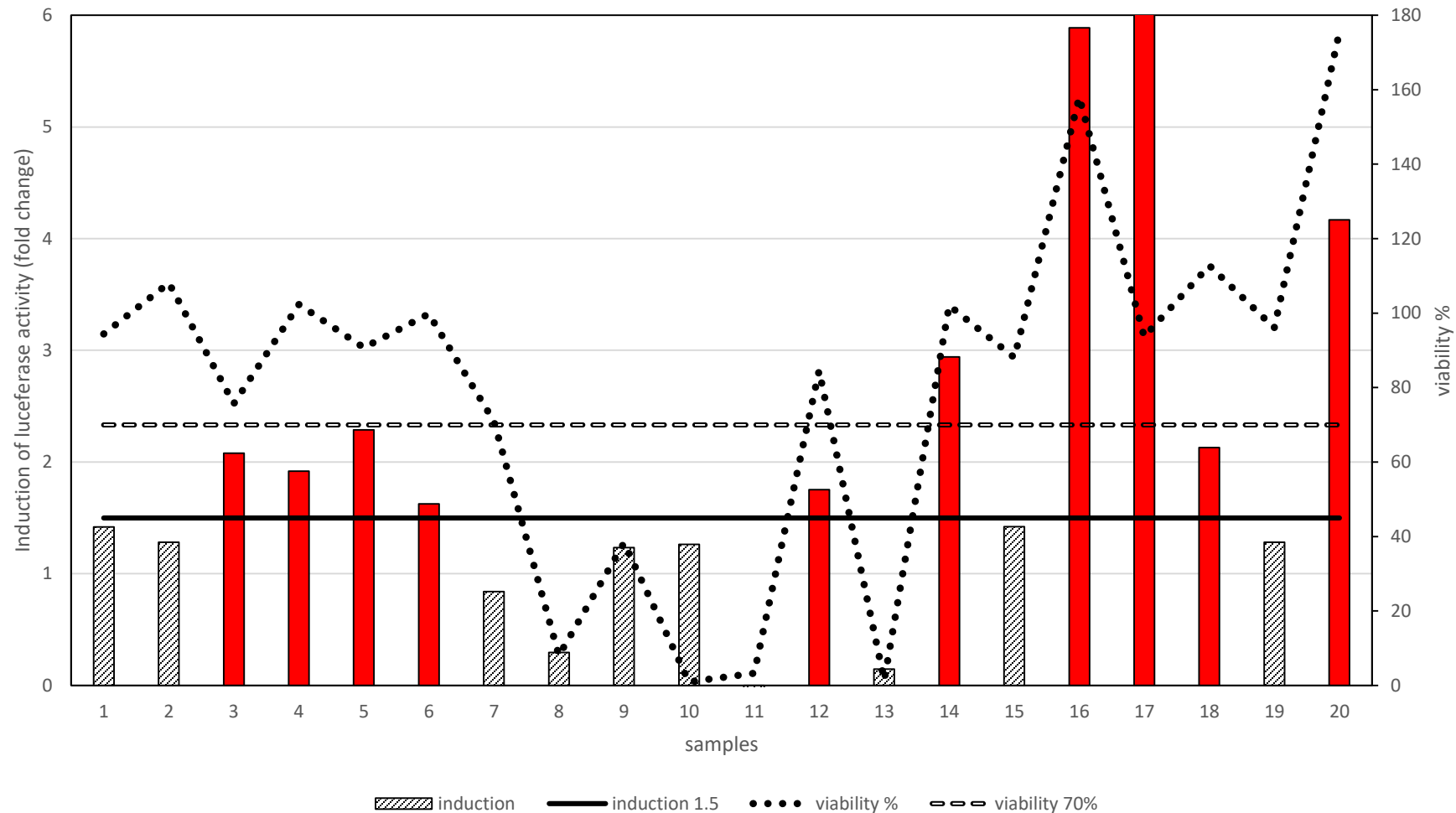
- ≥ 1.5 luciferase induction
- $\geq 70\%$ viability

**NONSENSITIZING
POTENTIAL**

- <1.5 luciferase induction
- $\geq 70\%$ viability

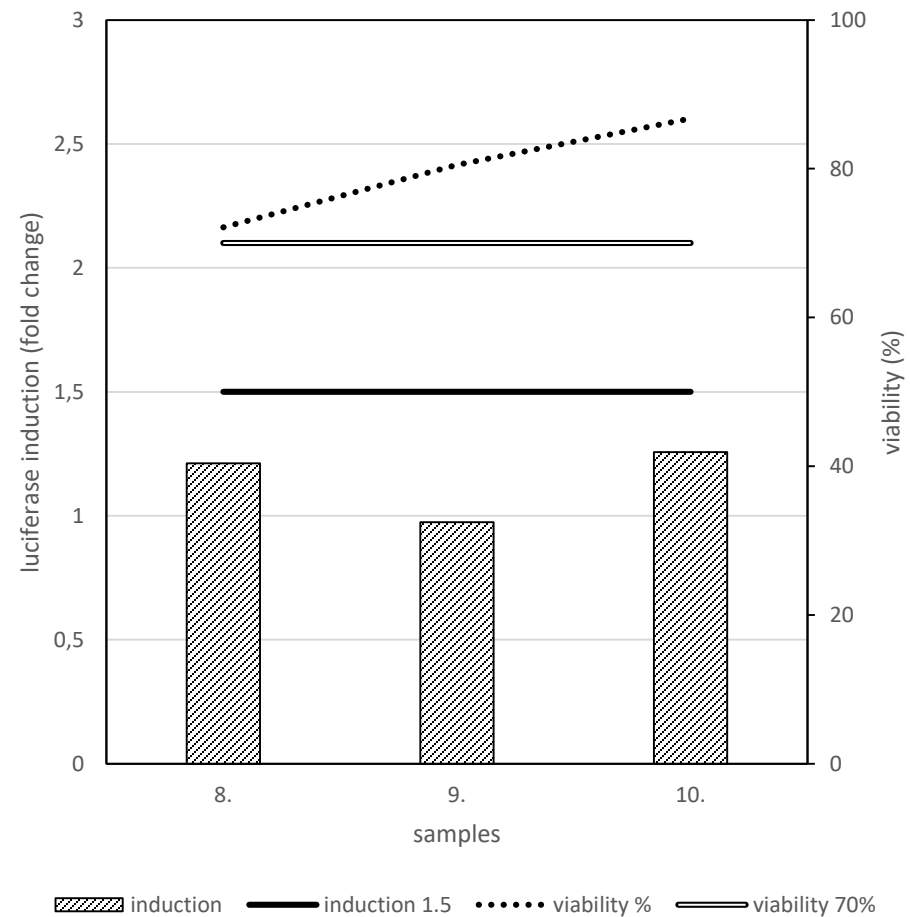


Sensitizing potential in 100% extract:
Samples 3, 4, 5, 6, 12, 14, 18 (weak)
16, 17, 20 (strong)
Cytotoxic effect in 100% extract:
Samples 8, 9, 10, 11, 13

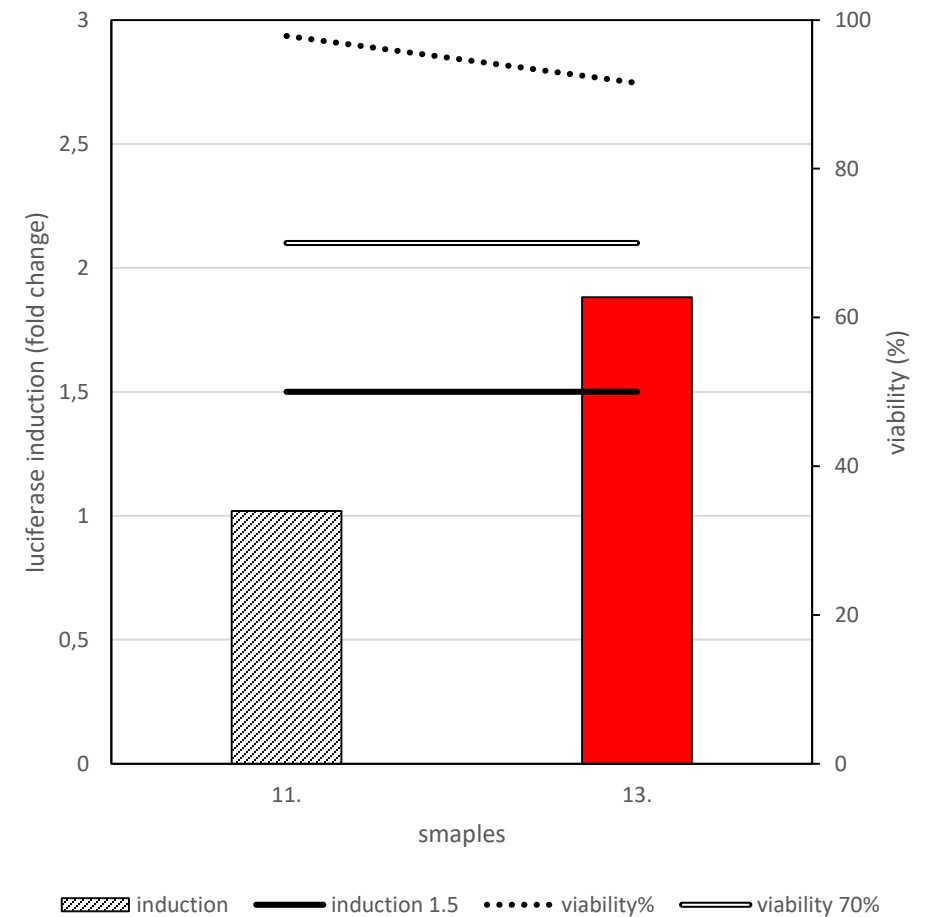




75% dilution



1% dilution



YES/ YAS



- ❑ Two genetically modified yeast strains *Saccharomyces cerevisiae*, identify compounds with potential interact with human estrogen and androgen receptors
- ❑ Transformed with plasmid carrying reporter gene encoding β -galactosidase and estrogen (YES) or androgen (YAS) responsive elements
- ❑ Enzyme β -galactosidase converts the yellow substrate into red product quantified on spectrophotometer (Eon)
- ❑ 96 well plates, 48 hours
- ❑ Evaluation:

**ESTROGENIC
EFFECT**

**ANTI
ESTROGENI
C EFFECT**

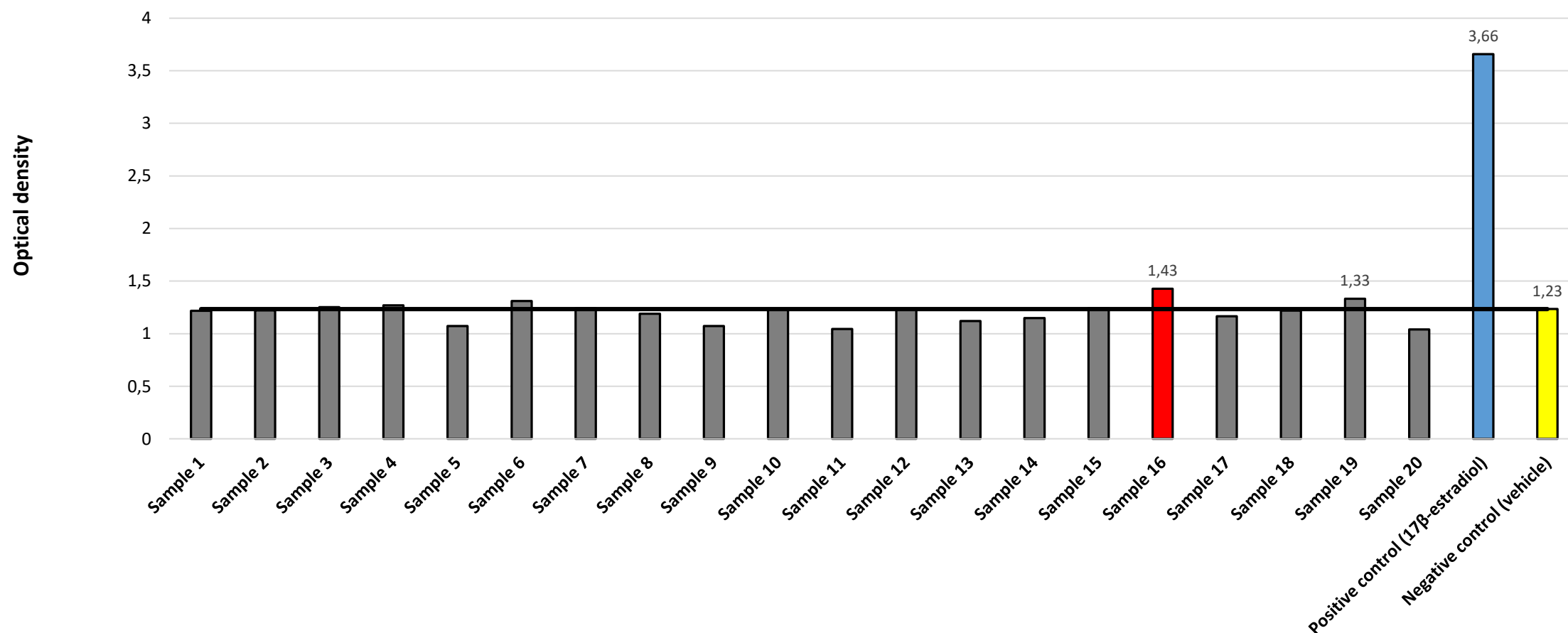
**ANDROGENIC
EFFECT**

**ANTI
ANDROGENIC
EFFECT**



YES/ YAS

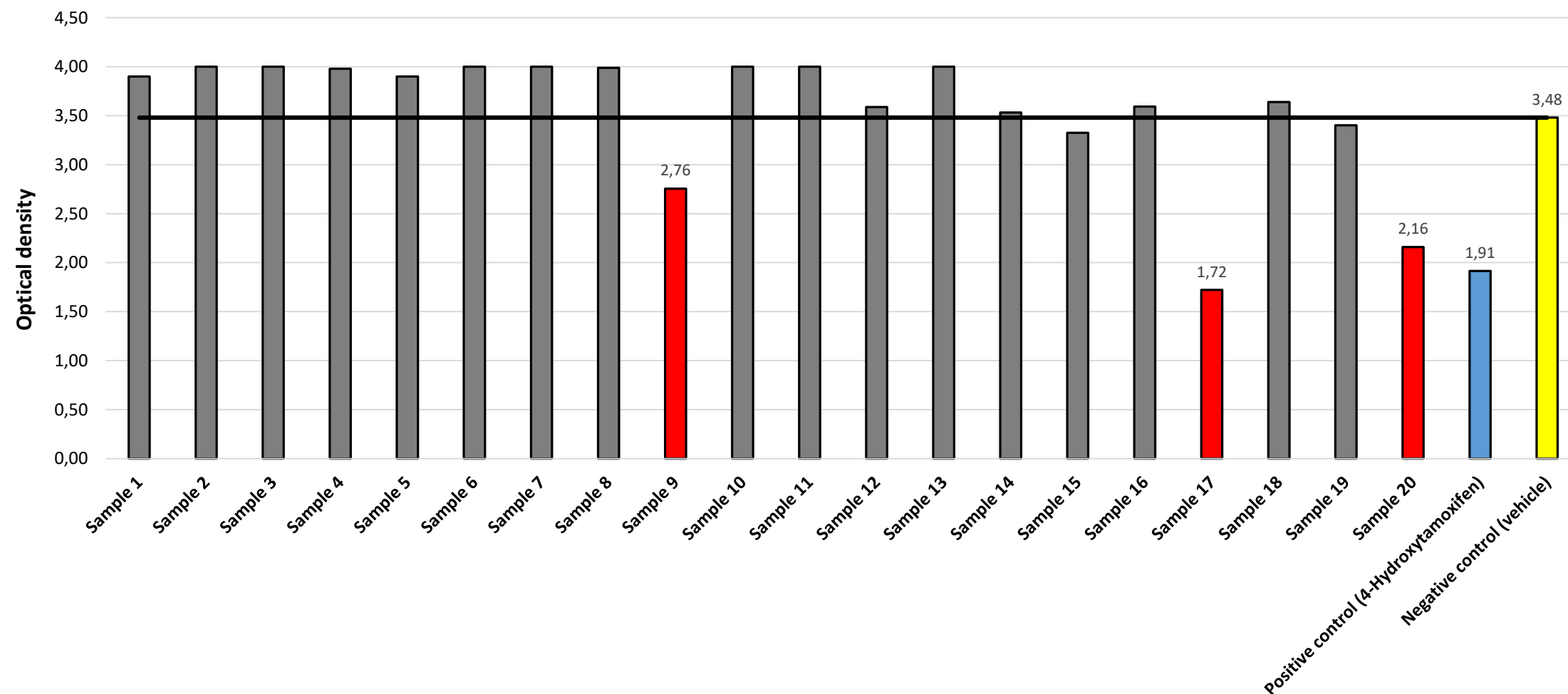
Agonistic activity on human estrogen receptor α :
sample 16 (weak)





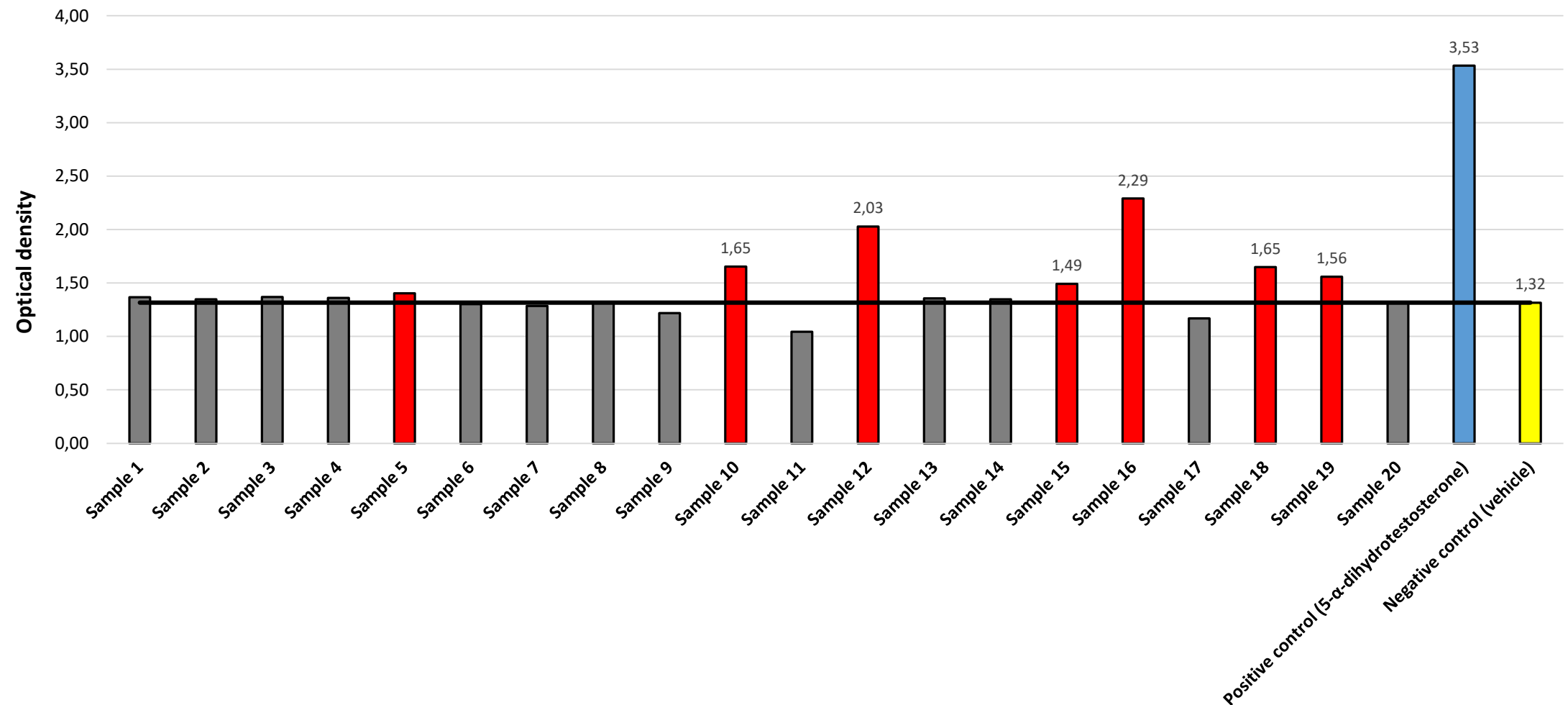
YES/ YAS

Antagonistic activity on human estrogen receptor α :
samples 9 (weak), 17, 20 (strong)





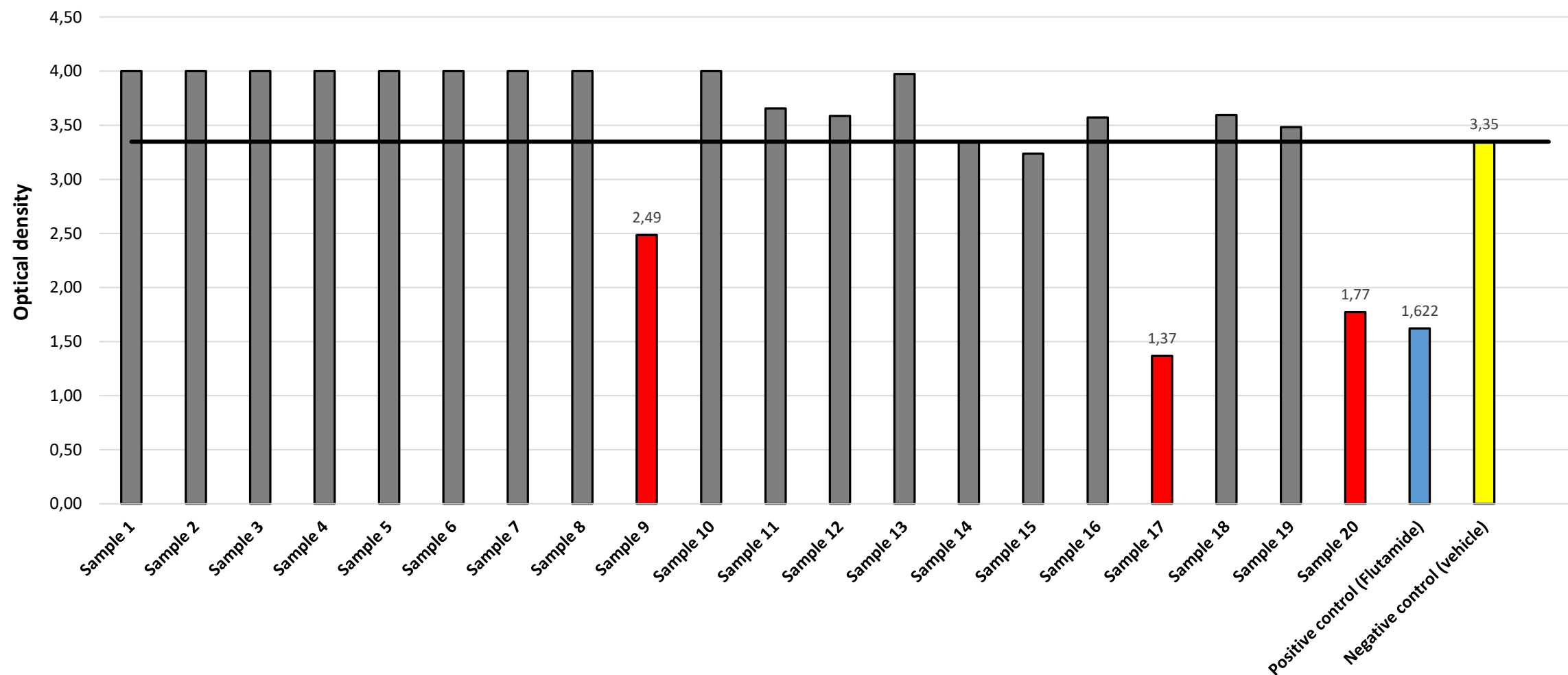
Agonistic activity on human androgen receptor:
Samples 16, 12 (strong), 10, 18 (weak),
5, 15, 19 (borderline)





YES/ YAS

Antagonistic activity on human androgen receptor:
Samples 17, 20 (strong), 9 (weak)





Effect:

* weak

** mild/medium

*** strong

No.	Made of	Colour	Cytotoxicity	DPRA	LuSens	YES	YAS
1	100% silicone	blue					
2	100% silicone	violet					
3	100% silicone	red			*		
4	100% silicone	purple			*		
5	100% silicone, ABS	violet			*		*
6	100% silicone	purple			*		
7	Thermoplastic rubber	pink					
8	Styrene-based elastomer	violet					
9	ABS	red				*	*
10	Polyester terephthalate, ABS	violet					*
11	polyisopren/ latex	nude	***	***			
12	PVC	nude			*		***
13	100% latex	black	***	***	*		
14	PVC (Jelly)	transparent	***		*		
15	silicone	green					*
16	PVC (Jelly)	purple			***	*	***
17	ABS	pink	***	*	***	***	***
18	PVC	nude	*		*		*
19	PVC	nude					*
20	ABS	green			**	**	**

Conclusions

- ❑ Colours (no ingredient information on package)
- ❑ Material (latex, PVC, ABS)
- ❑ Plasticizers (phtalates - DBT, TINTM; bisfenols, etc.)
- ❑ Optimization of extracts for medical devices
(not yet validated for sensitization tests and endocrine disruption test)





Conclusions

- ❑ Battery of selected in vitro tests was feasible – recommended for testing and monitoring to ensure better health safety of consumers
- ❑ These products should be recommended for only **occasional** use and short periods of time



A background image showing several glass petri dishes and a test tube with a red liquid drop, set against a colorful gradient background.

Thank you for your attention

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