

Metz et al.:

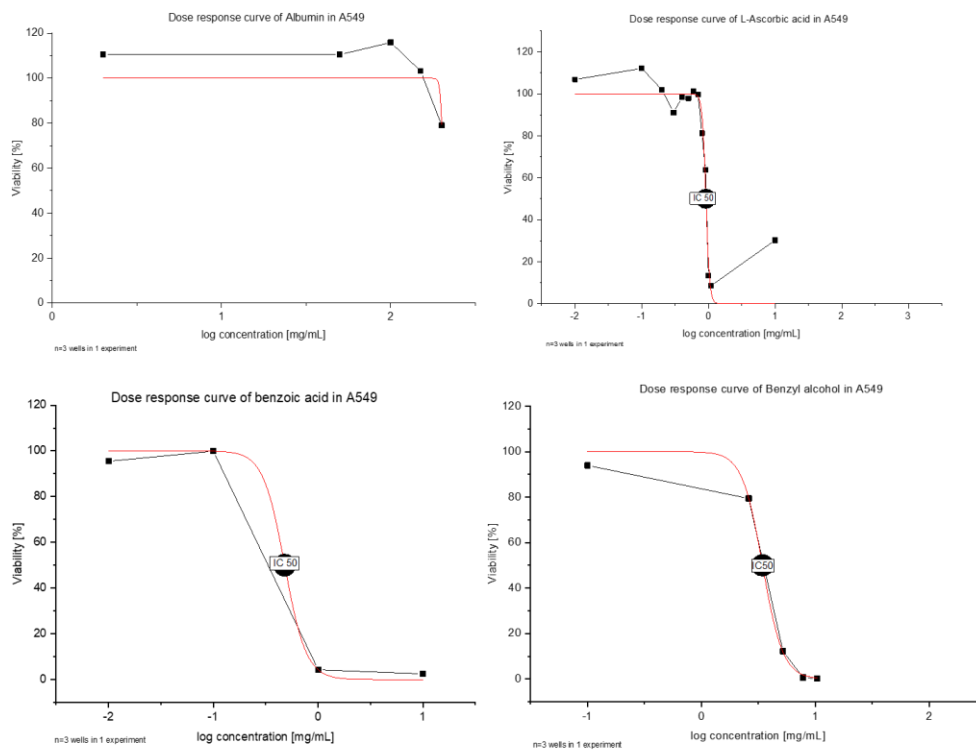
Safety Assessment of Excipients (SAFE) for Orally Inhaled Drug Products

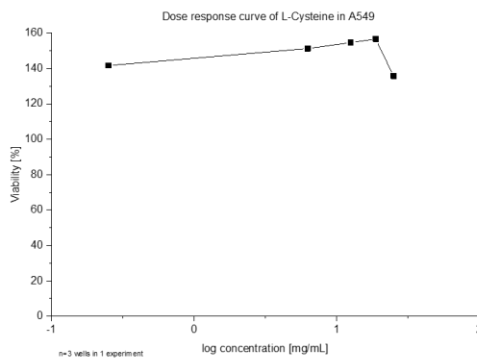
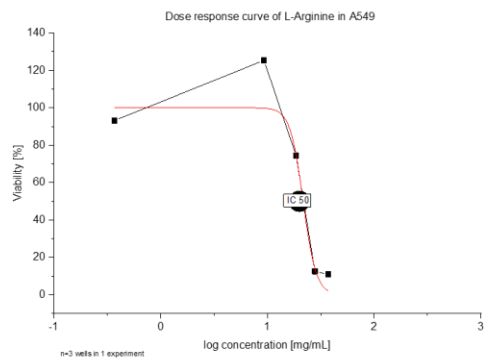
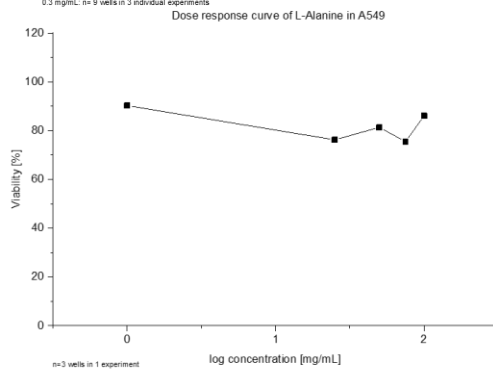
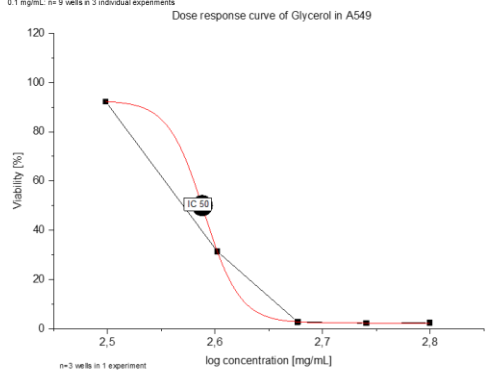
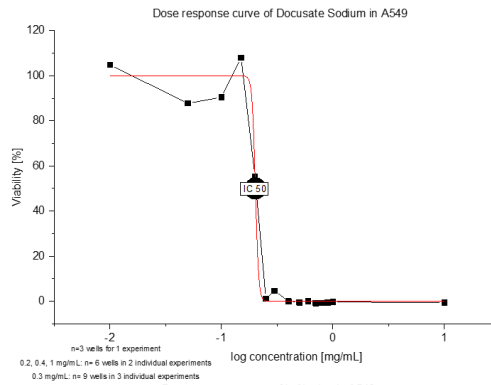
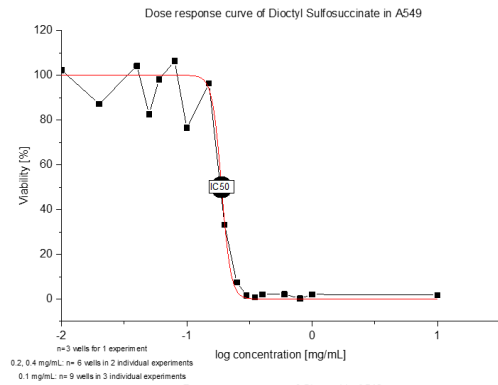
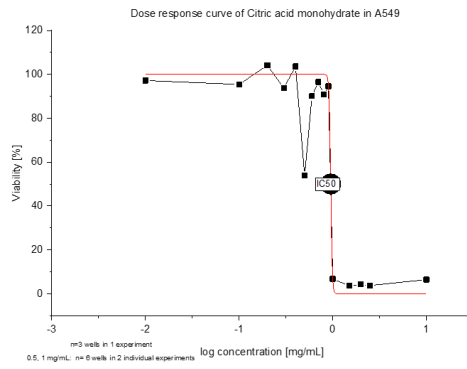
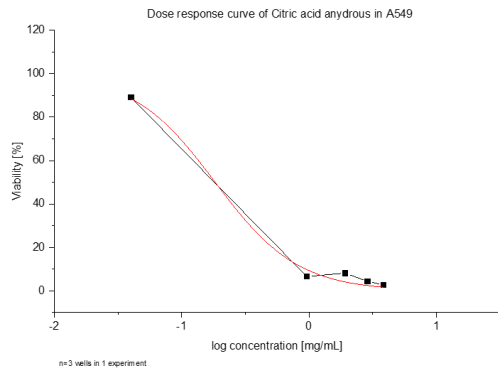
Supplementary Data

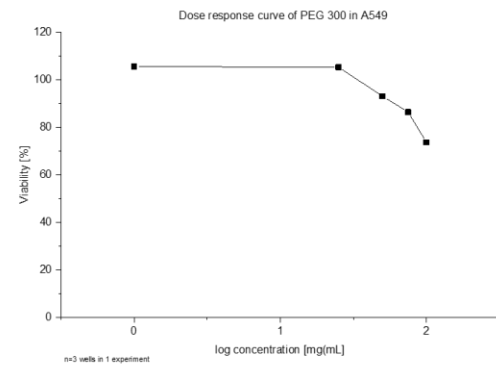
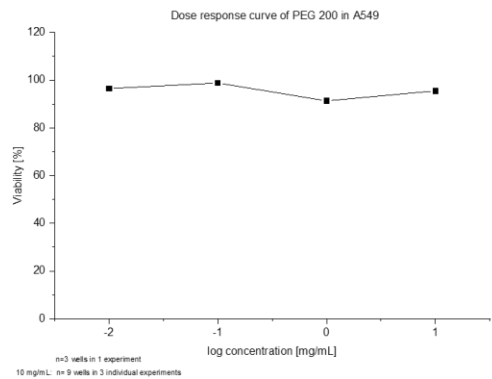
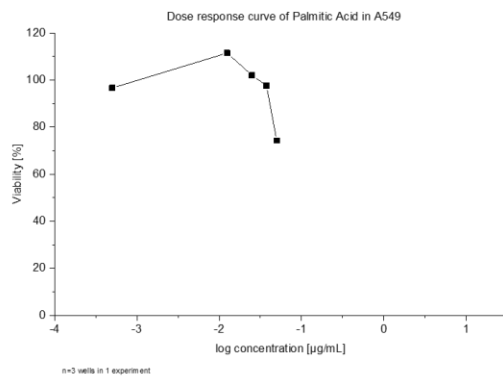
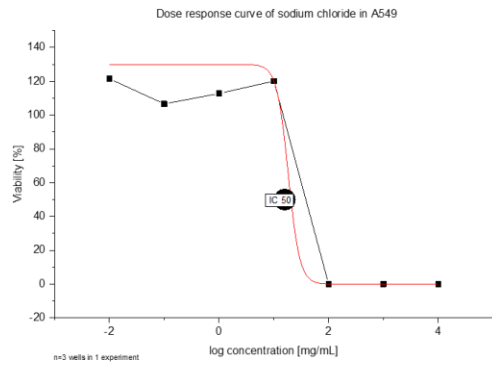
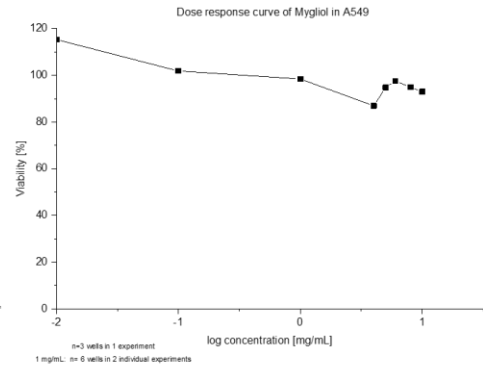
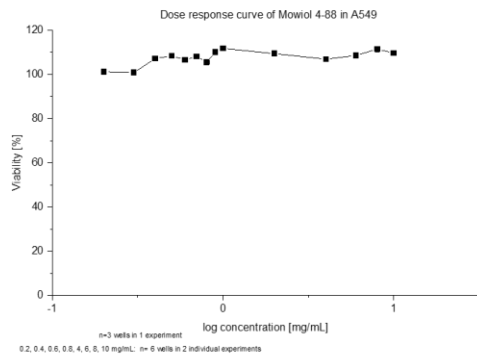
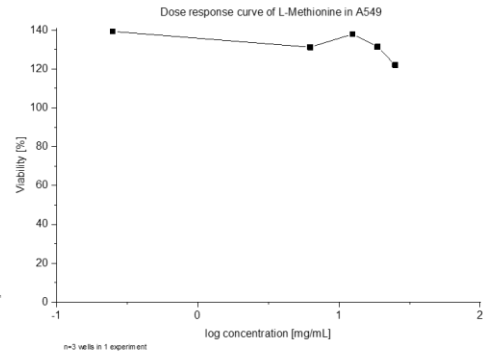
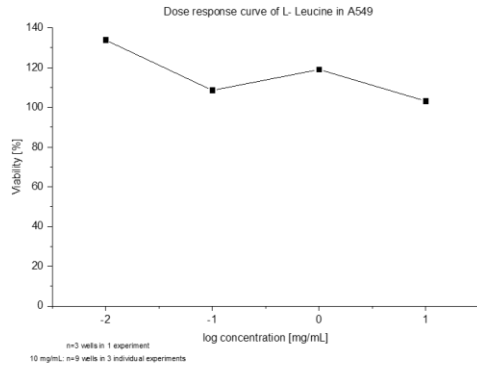
Dose response curves for the IC50 calculation *in vitro*

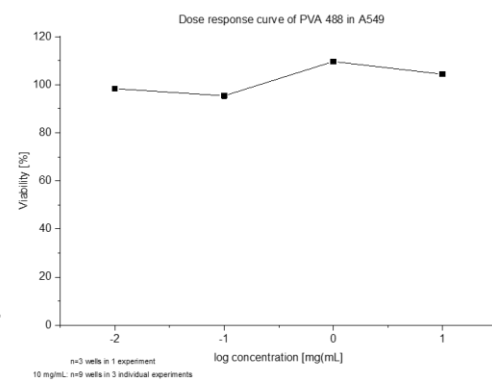
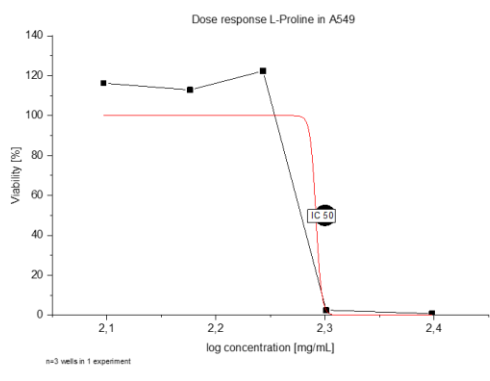
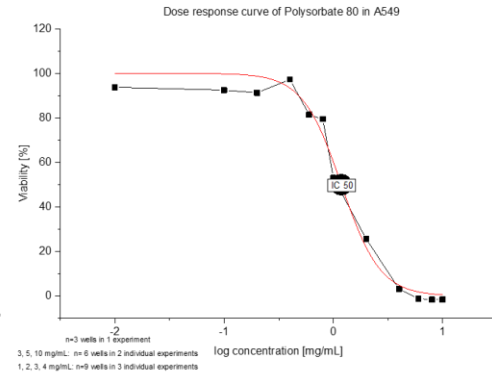
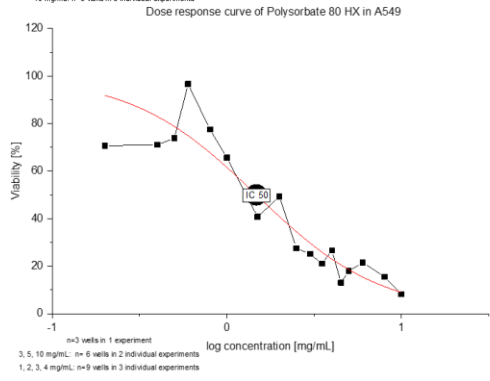
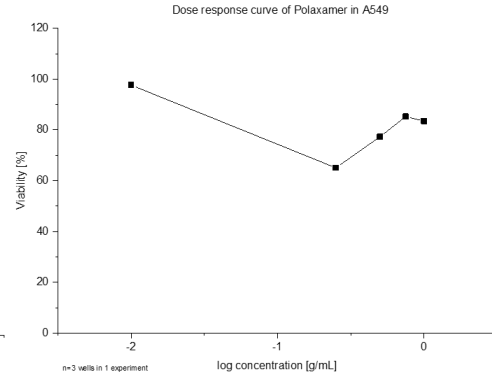
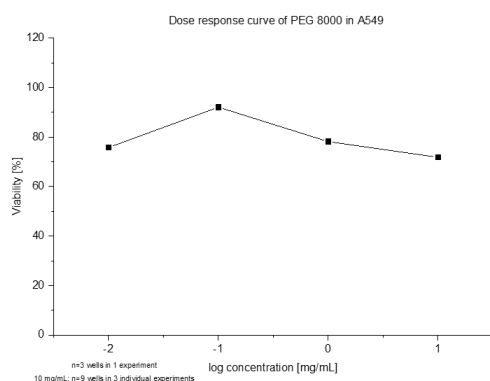
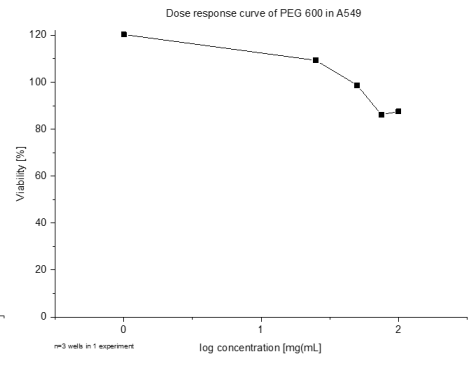
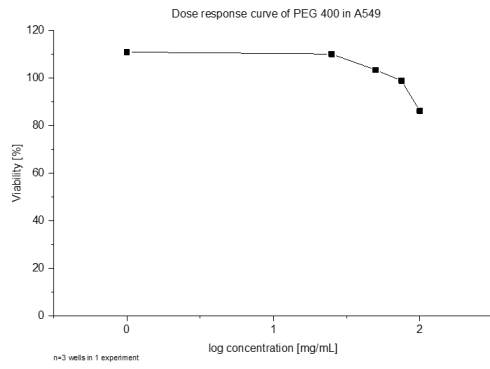
As a general survey, Fig. S1 (A549) and Fig. S2 (Calu-3) summarise the dose response curves of the tested excipients. By testing different concentrations of the test substances, the cell viability was determined via MTT assays to limit the range of the IC50. Subsequently, the curves were generated with the software Origin®Pro 2019 by applying sigmoidal fits which were used for the IC50 calculation *in vitro*. The description “n=3 wells in 1 experiment” explains that the mean of three wells per concentration is shown. Some experiments were applied to confirm the data which increases the number of wells by 3 for each additional experiment.

Dose response with IC50 calculation in A549 cells:









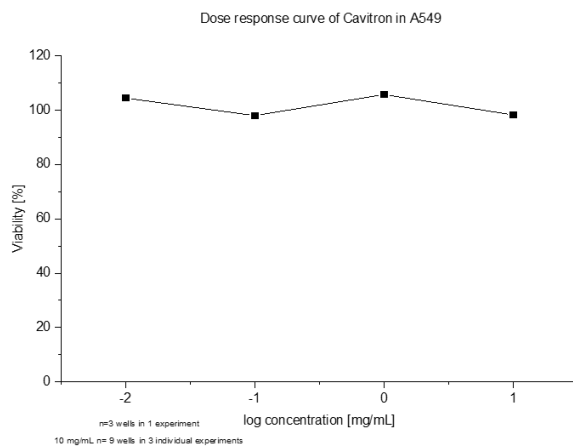
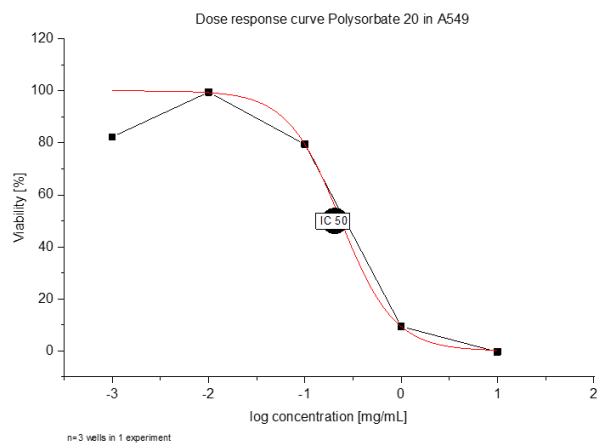
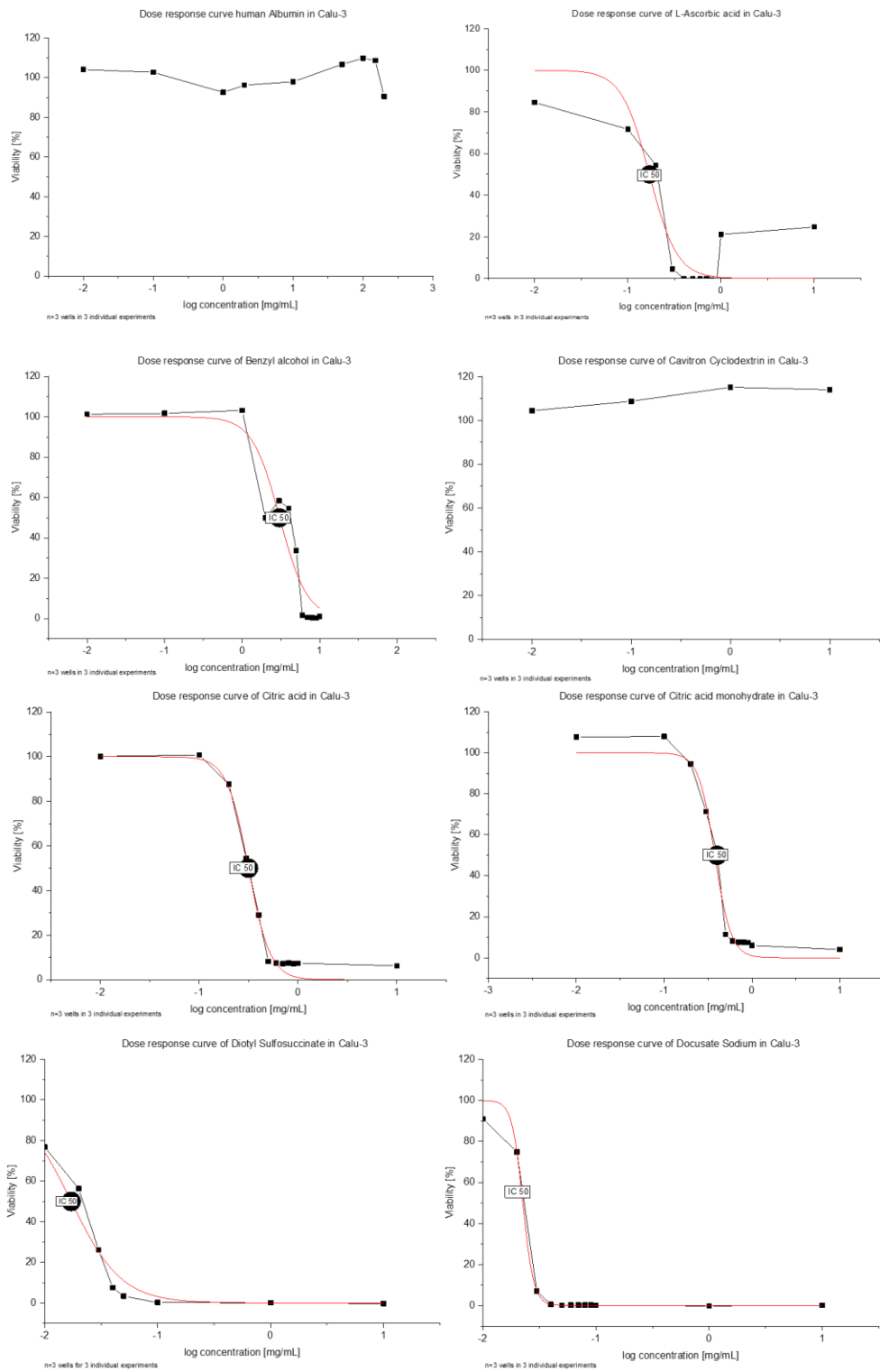
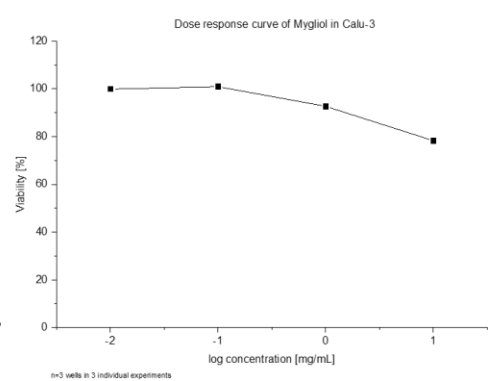
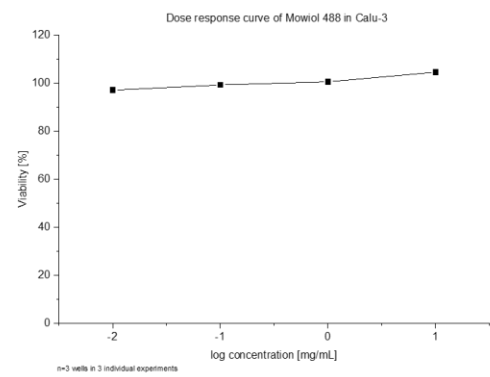
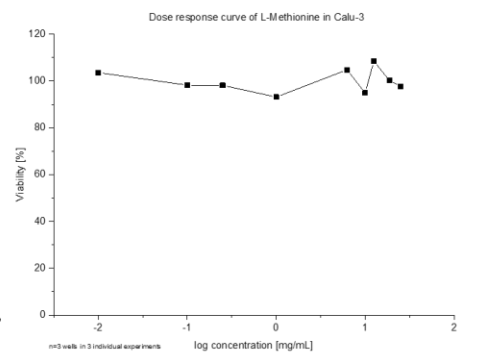
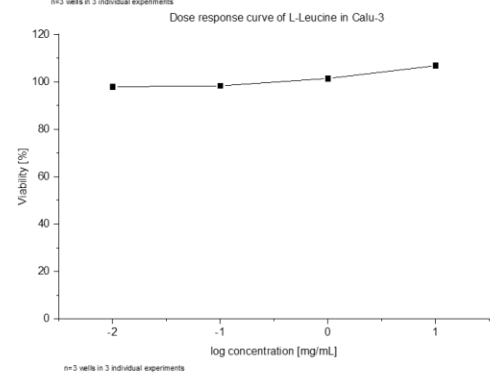
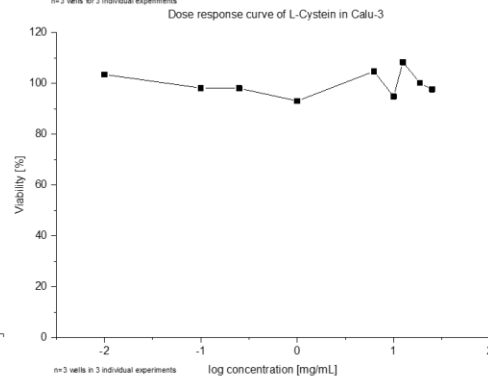
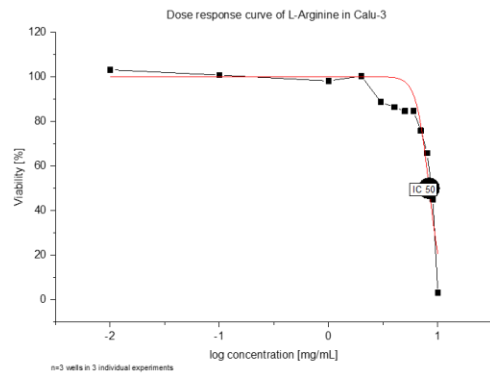
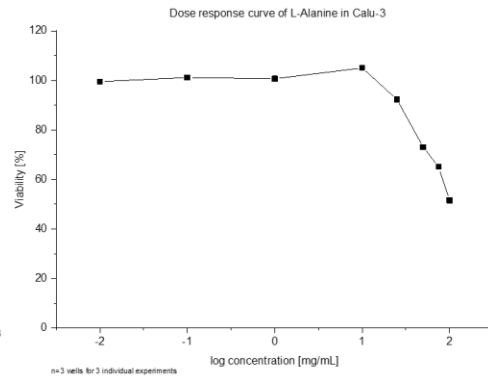
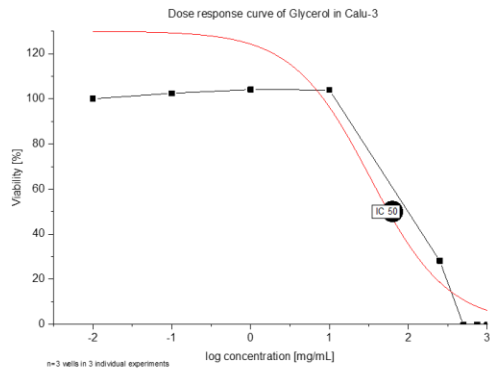
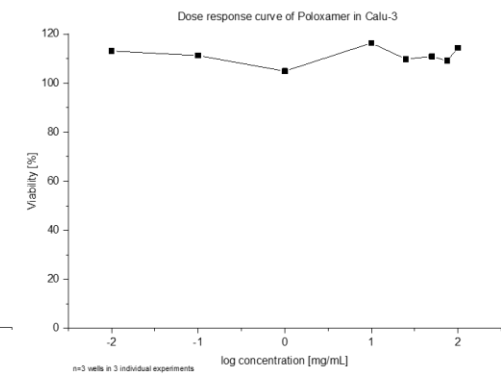
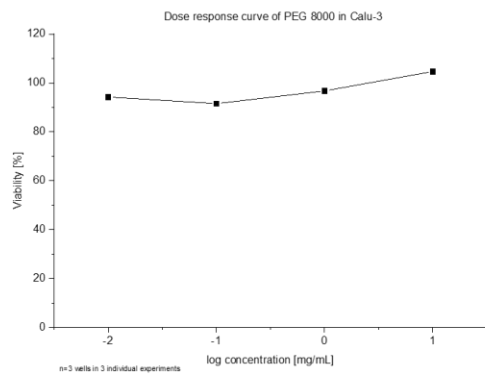
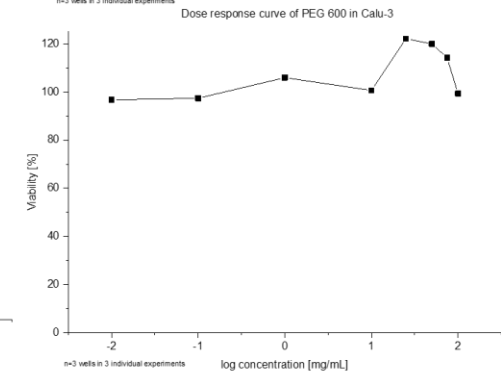
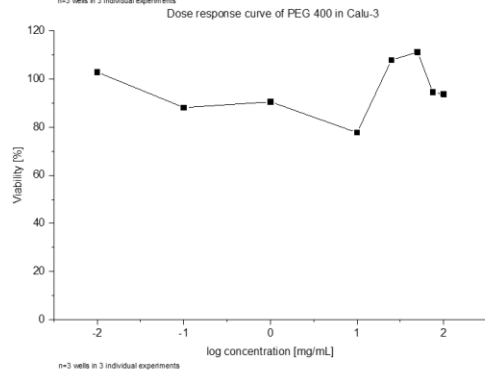
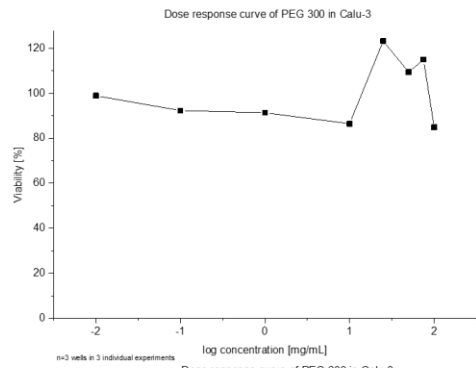
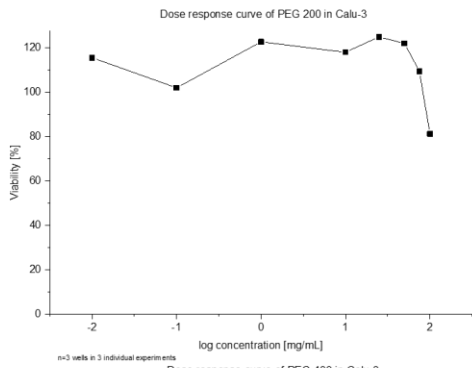
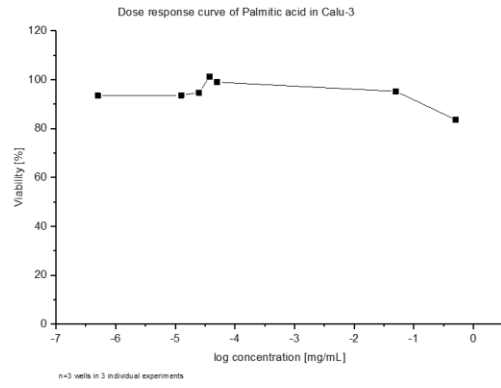
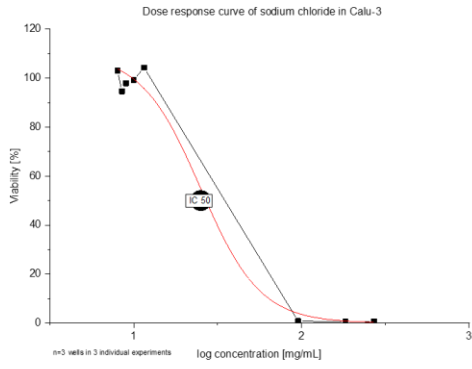


Fig. S1: Dose response and IC50 calculation of different excipients tested in A549 cells.

Dose response curves with IC50 calculation in Calu-3 cells:







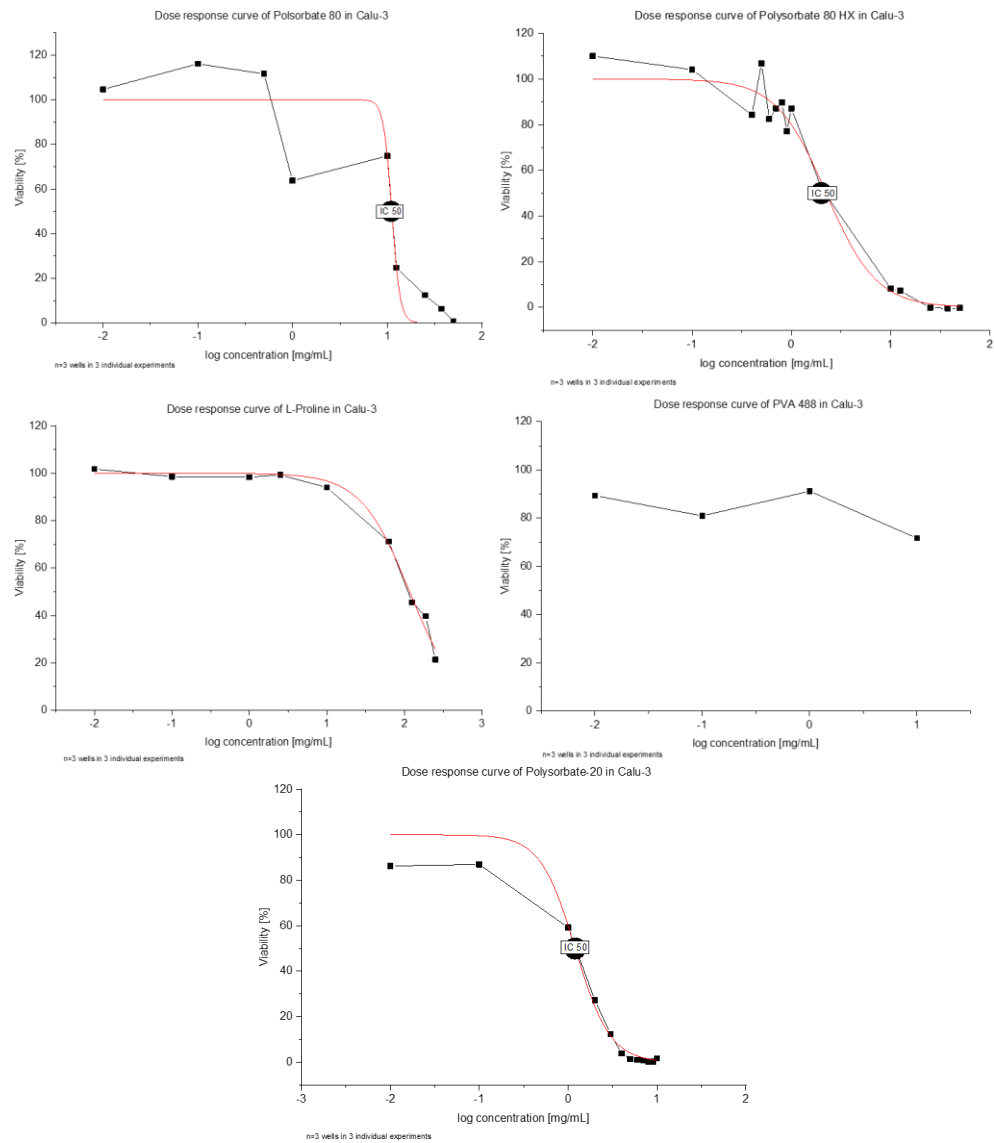


Fig. S2: Dose response and IC50 calculation of different excipients tested in Calu-3 cells.

Comparison between IC50 and GHS classification (LD50)

Tab. S1 lists the tested excipients with the corresponding GHS classification based on the pulmonary – or rather oral - LD50 values. This classification was used initially for the IVIVC, but was rejected due to the recalculation of the corresponding concentration applied *in vivo*.

Tab. S1: List of excipients with their associated oral and pulmonary GHS classification based on the related LD50

Substance	CAS number	LD50 <i>in vivo</i> oral	GHS classification acute toxicity oral	LD50 <i>in vivo</i> pulmonal	GHS classification acute toxicity pulmonal
Albumin from human serum	70024-90-7 FDA:9048468	GHS: Pre-Registration, no classification available GESTIS: no toxicity data available Handbook of pharm. Excipients: >12.5 g/kg (rat, monkey) ICE: no toxicity data available	Category 5	Pre-Registration, no classification available GESTIS: no toxicity data available Handbook of pharm. Excipients: no toxicity data available ICE: no toxicity data available	not available missing LD50
Benzoic acid	65-85-0	GHS: 2250 mg/kg bw (mouse) GESTIS: 1700 mg/kg Handbook of pharm. Excipients: 2 g/kg (cat, dog), 1.94 (mouse, rat) ICE: [1700-2742 mg/kg], n=5, median=2360 mg/kg	Category 4-5	GHS: > 12200 mg/m ³ air (rat, 4h, inhalation: dust) GESTIS: no toxicity data available Handbook of pharm. Excipients: no toxicity data available ICE: no toxicity data available	Category 5
Benzyl alcohol	100-51-6	GHS: 1.55 mL/kg bw (rat) GESTIS: 1230 mg/kg Handbook of pharm. Excipients: 1.36 g/kg (mouse), 1.23 g/kg (rat) ICE: [1230-3120 mg/kg], n=3, median=1230 mg/kg	Category 4	GHS: >4178 mg/m ³ air (rat, 4h, inhalation: aerosol) GESTIS: > 4178 mg/L (4 h, inhalation: aerosol) Handbook of pharm. Excipients: no toxicity data available ICE: no toxicity data available	Category 1 (gases classification) Category 5 (vapours classification) Category 5 (dusts classification)
Citric acid monohydrate	5949-29-1	GHS: no toxicity data available GESTIS: no toxicity data available Handbook of pharm. Excipients: no toxicity data available ICE: no toxicity data available	not available missing LD50	GHS: no toxicity data available GESTIS: no toxicity data available Handbook of pharm. Excipients: no toxicity data available ICE: no toxicity data available	not available missing LD50
Citric acid anhydrous	77-92-9	GHS: Pre-Registration, no classification available GESTIS: 3000 mg/kg (GESTIS) Handbook of pharm. Excipients: 5.04 g/kg (mouse), 3.0 g/kg (rat) ICE: [3000-11700 mg/kg], n=5, median=6730 mg/kg	Category 5	GHS: Pre-Registration, no classification available GESTIS: no toxicity data available Handbook of pharm. Excipients: no toxicity data available ICE: no toxicity data available	not available missing LD50
Docosate sodium	577-11-7	GHS: > 3 000 mg/kg bw (rat) GESTIS: no toxicity data available Handbook of pharm. Excipients: 2.64 g/kg (mouse), 1.9 g/kg (rat)	Category 5	GHS: 20 mg/L (rat, 96h, inhalation: aerosol) GESTIS: no toxicity data available Handbook of pharm. Excipients: no	Category 2 (gases classification) Category 4

Substance	CAS number	LD50 <i>in vivo</i> oral	GHS classification acute toxicity oral	LD50 <i>in vivo</i> pulmonal	GHS classification acute toxicity pulmonal
		ICE: [1320-4200 mg/kg], n=9, median=2100 mg/kg		toxicity data available ICE: no toxicity data available	(vapours classification) Category 5 (dusts classification)
Glycerol	56-81-5	GHS: 27 mg/kg bw (rat) GESTIS: 12600 mg/kg (rat) Handbook of pharm. Excipients: 7.75 g/kg (guinea pig), 4.1 g/kg (mouse), 27 g/kg (rabbit), 5.57-12.6 g/kg (rat) ICE: [5570-27650 mg/kg], n=12, median=17102.5 mg/kg	Category 2 (GHS) Category 5	GHS: 4655 mg-min/L (rat, 7h, inhalation: vapour) GESTIS: no toxicity data available Handbook of pharm. Excipients: no toxicity data available ICE: no toxicity data available	Category 5
L(+)-Ascorbic acid	50-81-7	GHS: Pre-Registration, no classification available GESTIS: 11900 mg/kg (rat) Handbook of pharm. Excipients: 3.37 g/kg (mouse), 11.9 g/kg (rat) ICE: [11900-11900 mg/kg], n=2, median=11900 mg/kg	Category 5	GHS: Pre-Registration, no classification available GESTIS: no toxicity data available Handbook of pharm. Excipients: no toxicity data available ICE: no toxicity data available	not available missing LD50
L-Alanine	56-41-7	GHS: > 5110 mg/kg bw (rat) GESTIS: no toxicity data available Handbook of pharm. Excipients: no toxicity data available ICE: no toxicity data available	Category 5	GHS: no toxicity data available GESTIS: no toxicity data available Handbook of pharm. Excipients: no toxicity data available ICE: no toxicity data available	not available missing LD50
L-Arginine	74-79-3	GHS: 5110 mg/kg bw (rat) GESTIS: no toxicity data available Handbook of pharm. Excipients: no toxicity data available ICE: no toxicity data available	Category 5	GHS: no toxicity data available GESTIS: no toxicity data available Handbook of pharm. Excipients: no toxicity data available	not available missing LD50
L-Cysteine	52-90-4	GHS: 5.85 g/kg bw (rat) GESTIS: no toxicity data available Handbook of pharm. Excipients: no toxicity data available ICE: [1890-1890 mg/kg], n=2, median=1890 mg/kg	Category 5	GHS: no toxicity data available GESTIS: no toxicity data available Handbook of pharm. Excipients: no toxicity data available ICE: no toxicity data available	not available missing LD50
L-Methionine	63-68-3	GHS: > 10 000 mg/kg bw (rat) GESTIS: 36000 mg/kg (rat) Handbook of pharm. Excipients: 36 g/kg (rat) ICE: [36000-36000 mg/kg], n=2, median=36000 mg/kg	Category 5	GHS: > 5.25 mg/L air (analytical) (rat, 4h inhalation: dust) GESTIS: no toxicity data available Handbook of pharm. Excipients: no toxicity data available ICE: no toxicity data available	Category 5
L-Proline	147-85-3	GHS: > 5110 mg/kg bw (rat) GESTIS: no toxicity data available Handbook of pharm. Excipients: no toxicity data available ICE: no toxicity data available	Category 5	GHS: no toxicity data available GESTIS: no toxicity data available Handbook of pharm. Excipients: no toxicity data available ICE: no toxicity data available	not available missing LD50
Palmitic acid	57-10-3	GHS: >5 000 mg/kg bw (rat) GESTIS: > 10000 mg/kg (rat)	Category 5	GHS: > 0.162 mg/L (rat, 4h, inhalation: vapour)	Category 1-5

Substance	CAS number	LD50 <i>in vivo</i> oral	GHS classification acute toxicity oral	LD50 <i>in vivo</i> pulmonal	GHS classification acute toxicity pulmonal
		Handbook of pharm. Excipients: no toxicity data available ICE: 10000 mg/kg		GESTIS: no toxicity data available Handbook of pharm. Excipients: no toxicity data available ICE: no toxicity data available	
Poloxamer 188 (Kolliphor® 188)	/	GHS: Pre-Registration, no classification available GESTIS: no toxicity data available Handbook of pharm. Excipients: 15 g/kg (mouse), 9.4 g/kg (rat) ICE: no toxicity data available	Category 5	GHS: Pre-Registration, no classification available GESTIS: no toxicity data available Handbook of pharm. Excipients: no toxicity data available ICE: no toxicity data available	not available missing LD50
Polyethylene glycol 200 (PEG 200)	25322-68-3	GHS: Pre-Registration, no classification available GESTIS: no toxicity data available Handbook of pharm. Excipients: 34 g/kg (mouse), 28.0 g/kg (rat), 19.9 g/kg (rabbit) ICE: no toxicity data available	Category 5	GHS: Pre-Registration, no classification available GESTIS: no toxicity data available Handbook of pharm. Excipients: no toxicity data available ICE: no toxicity data available	not available missing LD50
Polyethylene glycol 300 (PEG 300)		GHS: Pre-Registration, no classification available GESTIS: no toxicity data available Handbook of pharm. Excipients: 27.5 g/kg (rat), 17.3 g/kg (rabbit), 19.6 g/kg (guinea pig) ICE: no toxicity data available	Category 5		
Polyethylene glycol 400 (PEG 400)		GHS: Pre-Registration, no classification available GESTIS: no toxicity data available Handbook of pharm. Excipients: 28.9 g/kg (mouse), 26.8 g/kg (rabbit), 15.7 g/kg (guinea pig) ICE: no toxicity data available	Category 5		
Polyethylene glycol 600 (PEG 600)		GHS: Pre-Registration, no classification available GESTIS: no toxicity data available Handbook of pharm. Excipients: 47 g/kg (mouse), 38.1 g/kg (rat) ICE: no toxicity data available	Category 5		
Polysorbate 80 (Tween-80)		9005-65-6	GHS: Pre-Registration, no classification available GESTIS: no toxicity data available Handbook of pharm. Excipients: 25 g/kg (mouse) ICE: no toxicity data available		
Polysorbate 80 (HX2)					
Polysorbate 20	9005-64-5	GHS: 36700 mg/kg bw (rat)	Category 5	GHS: > 5.1 mg/L (rat, 4h, inhalation:	Category 2

Substance	CAS number	LD50 <i>in vivo</i> oral	GHS classification acute toxicity oral	LD50 <i>in vivo</i> pulmonal	GHS classification acute toxicity pulmonal
(Tween 20)		GESTIS: no toxicity data available Handbook of pharm. Excipients: 37 g/kg (rat), 18 g/kg (hamster) ICE: no toxicity data available		aerosol) GESTIS: no toxicity data available Handbook of pharm. Excipients: no toxicity data available ICE: no toxicity data available	(gases classification) Category 3-5 (vapours classification) Category 5 (dusts classification)
Sodium chloride	7647-14-5	GHS. 3550 mg/kg be (rat) GESTIS: 3000 mg/kg (rat) Handbook of pharm. Excipients: 4.09 g/kg (mouse), 3 g/kg (rat) ICE: no toxicity data available	Category 5	GHS: >42 mg/l (rat, 1h, inhalation: aerosol) GESTIS: no toxicity data available Handbook of pharm. Excipients: no toxicity data available ICE: no toxicity data available	Category 2 (gases classification) Category 5 (vapours classification) Category 5 (dusts classification)

Correlation of the IC50 of A549 and Calu-3 cells

For the evaluation of the cellular variations between A549 and Calu-3 a regression analysis was performed including the IC50 of the substances for which an IC50 calculation was possible (benzyl alcohol, citric acid monohydrate, citric acid anhydrous, docusate Sodium, L(+)-Ascorbic acid, L-Arginine, Polysorbate 80 (Tween-80), Polysorbate 80 (HX2) and Polysorbate 20 (Tween 20)). A positive slope of the regression line of 0.88 ± 0.36 was obtained by performing a correlation analysis with a R^2 of 0.46 (Fig.S3). In sum, a point to point correlation is not given.

When comparing both data sets (IC50 A549 & Calu-3) by their *in vitro* hazard class (Sauer et. al 2013, A549), we find similar results (11/15 hits) and no preference for lower/higher IC50 values for A549/Calu-3 (Tab. S2).

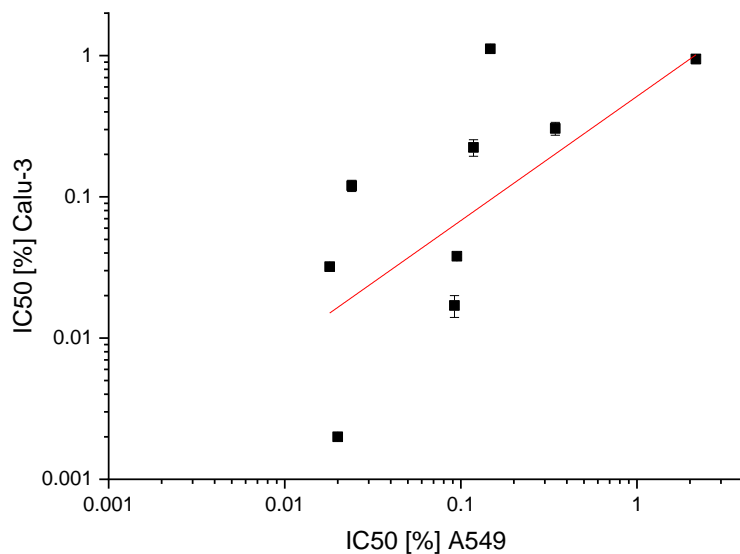


Fig. S3: Regression analysis of IC50 [%] *in vitro* in Calu-3 cells vs. IC50 [%] in A549

A linear regression was performed with the Software Origin®Pro 2019. The values for the regression analysis are: Slope: 0.88 ± 0.36 , Pearson R: 0.68; coefficient of determination (COD, R^2): 0.46; Correlation R^2 : 0.38.

Tab. S2: Comparison of IC50 data

Substance	IC50			
	<i>In vitro</i> hazard class			
	1	2	3	4
	< 0.1 mg/mL	0.1-1 mg/mL	1-10 mg/mL	> 10 mg/mL
	< 0.01%	0.01-0.1%	0.1%-1%	>1%
Albumin from human serum				A549 & Calu-3
Benzyl alcohol			A549 & Calu-3	
Citric acid monohydrate		A549 & Calu-3		
Citric acid anhydrous		A549 & Calu-3		
Docusate Sodium	Calu-3	A549		
L(+)-Ascorbic acid		A549 & Calu-3		
L-Alanine				A549 & Calu-3
L-Arginine		Calu-3	A549	
Polyethylene glycol 200 (PEG 200)				A549 & Calu-3
Polyethylene glycol 300 (PEG 300)				A549 & Calu-3
Polyethylene glycol 400 (PEG 400)				A549 & Calu-3
Polyethylene glycol 600 (PEG 600)				A549 & Calu-3
Polysorbate 80 (Tween-80)			A549	Calu-3
Polysorbate 80 (HX2)			A549 & Calu-3	
Polysorbate 20 (Tween 20)		A549	Calu-3	
IC50 (Calu 3 > A549)	2			
IC50 (A549 > Calu-3)	2			
Same range	11			