Supplementary Material

Comprehensive review of epidemiological and animal studies on the potential carcinogenic effects of nicotine *per se*

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Supplementary Table 1. Survey of studies relevant to assess the potential of nicotine to cause cancer in animals. Shaded rows indicate studies with low-adequacy scores (≤2); narratives on these low-scoring studies are presented in Supplementary Table 4.

						Nicotine						Study adequacy						
Reference	Route of adminis-tration	Species	Strain	Sex	Group size: Nic./ Contr.	Nominal dose (mg/(kg × d))*	Regimen	Duration (months)	Nicotine effects	Comments	Conclusion on *per se* carcino-genicity by authors	Route	Group size†	Dose-response	Daily dose‡	Dura-tion¶	Qual-ity§	Overall adequacy score
Waldum et al. 1996	inhalation	Rat	Sprague-Dawley	F	N: 68 C: 34	0.4	20 h/d, 5 d/week	24	- numerical increase in overall tumor incidence (21/59 vs. 6/25 in nicotine vs. control groups) - numerical increase in pituitary tumors: 5/59 vs. 0/25 - no lung tumors in nicotine or control groups - no effect on lung neuroendocrine cell turnover - plasma nicotine: 130 ng/ml	- nicotine aerosol concentration: 0.5 mg/m^3 - nicotine dose estimated based on respiratory minute volume assuming full retention - body weight effects - insufficient statistical power due to intermediate sacrifices: 22 /7 rats for nicotine/control groups, resp., at termination	negative	+	-	-	+	+	+	4
Wilson & DeEds 1936; Wilson et al. 1938	oral	Rat	Albino	F+M	?	>33	up to 0.05% in food	10	- microscopic examination of organs revealed only little difference	- retarded body weight development - lower food consumption	negative	+	-	+	+	-	-	3
Toth 1982	oral	Mouse	Swiss	F+M	N: 100 C: 200	150	drinking water, 0.063% and 0.094% nicotine hydrochloride	30	- no difference in tumor incidence by nicotine - broad checking of organs and tissues	- no toxicity reported - no effect on survival - background lung tumor incidence approx. 20% - no actual difference between calculated doses per mouse	negative	+	+	+	+	+	+	6
Murphy et al. 2011	oral	Mouse	A/J	F	19	6	drinking water 0.2 NHT mg/ml	11	- no effect on lung tumor multiplicity - no effect of tumor size - urinary nicotine: 1300 ng/ml: cotinine: 4400 ng/ml - plasma nicotine: 0.4 ng/ml: cotinine: 19 ng/ml	- water consumption: 15 ml/week - no systematic investigation of all organs and tissues	negative	+	-	-	+	-	+	3

Supplementary Table 1 (continued)

Reference	Route of adminis-tration	Species	Strain	Sex	Group size: Nic./ Contr.	Nicotine			Nicotine effects	Comments	Conclusion on *per se* carcino-genicity by authors	Study adequacy						
						Nominal dose (mg/(kg × d))*	Regimen	Duration (months)				Route	Group size†	Dose-response	Daily dose‡	Dura-tion¶	Qual-ity§	Overall adequacy score
Hermann et al. 2014	oral	Mouse	C57/Bl6	?	3	20	Drinking water 0.1 mg/ml	18	- no effect on area of pancreatic interepithelial neoplasia lesions	- used to confirm earlier reports of no effects on pancreatic tumorigenesis and performed in parallel to studies with K-Ras mutant strains - some inconsistencies in reported materials and methods - drinking water was supplemented with 2% sucrose	negative	+	-	-	+	+	-	3
Nishikawa et al. 1992	oral	Hamster	Syrian Golden	F	30	2.5 ||	25 ppm in drinking water	9	- no effect of nicotine on pancreatic carcinogenesis	- no body weight effect due to nicotine treatment - histopathology was well conducted with serial sectioning in four pancreatic lobes	negative	+	-	-	+	-	+	3
Thompson et al. 1973	s.c.	Rat	Fischer	M	38/10	1	Nicotine was suspended in gelatin with the idea of sustained release over the day	22	- overall tumor incidences of 33% in control and 29% in nicotine-exposed groups - tumor patterns considered typical for the strain and age of the rats used - statistically significant increase in Leydig cell hyperplasia (although on a high age-related level)	- only 28 rats in the nicotine group and 6 rats in the control group survived the study (mostly technical reasons for deaths) - decreased body weight development by nicotine (approximately 15% at maximum) - large range of organs and tissues investigated - hematological and biochemical investigations included in this and parallel papers	negative	-	-	-	+	+	+	3
Yokohira et al. 2012	intra-tracheal instillation	Rat	F344	M	3-5	-	0.05, 0.1, 0.2 mg/rat, 3 to 9 times	7.5	- no tumors, - no proliferative changes in lungs, liver, kidneys - neutrophil accumulation, edema, fibrosis in lungs (no statistics)	- no dose dependency, but authors discuss impact on frequency of administration - no clear body weight or organ weight effects - infrequent instillation not considered to be a useful model for daily inhalation	-	-	-	+	-	-	+	2

Supplementary Table 1 (continued)

Reference	Route of adminis-tration	Species	Strain	Sex	Group size: Nic./ Contr.	Nicotine			Nicotine effects	Comments	Conclusion on *per se* carcino-genicity by authors	Study adequacy						
						Nominal dose (mg/(kg × d))*	Regimen	Duration (months)				Route	Group size†	Dose-response	Daily dose‡	Dura-tion¶	Qual-ity§	Overall adequacy score
Schoental & Head 1953	oral	Mouse	CBA	?	?	?	?	> 24	- no increased incidence of tumors	- only abstract available: no information on dosing and group sizes	negative	+	-	-	-	+	-	2
Truhaut & De Clercq 1961	oral	Rat	Wistar	F+M	N: 45 C: ?	?	drinking water	24	- 1/45 tumor (liver/intestine), none in controls	- unclear to what extent other organs and tissues were examined	negative	+	-	-	-	+	-	2
Schoental & Head 1953	dermal	Mouse	CBA	?	?	?	skin painting	> 24	- no increased incidence of tumors - 7/14 mice of mixed strains developed lung adenomas after exposure to a nicotine pyrolysate	- only abstract available: no information on dosing and group sizes	negative	+	-	-	-	+	-	2
Staemmler 1935; 1936	s.c.	Rat	?	F+M	N: 38 C: ?	0.3 up to 5	-	20	- hyperplasia and adenoma in adrenal medulla (12/30) - testicular atrophy	- doses vary among individual rats (0.3 mg/(kg × d) for those 2 rats with medulla adenoma) - duration in study varies among individual rats - no systematic investigation of all organs and tissues	positive	-	-	-	+	+	-	2
Yun & Kim 1938	s.c.	Guinea pig	-	F	?	?	0.3 mg on 5 days between study days 15 and 190	6	- no carcinogenic effect by nicotine - early medullar atrophy, later hypertrophy	- insufficient information on study details	n.a.	-	-	-	-	-	-	0
Hueper 1943	s.c.	Rat	?	F+M	60	33	10 mg, 5 d/week	8	- no nicotine-related carcinogenicity	- study was not planned for carcinogenesis testing, i.e. study duration relatively short due to high mortality - nicotine dose was gradually increased during study - partially severe morphological effects on various organs and tissues by the high level nicotine dosing - also s.c. administration to dogs, two survivors for 10 months: no	n.a.	-	-	-	+	-	-	1

Supplementary Table 1 (continued)

Reference	Route of administration	Species	Strain	Sex	Group size: Nic./ Contr.	Nicotine			Nicotine effects	Comments	Conclusion on *per se* carcinogenicity by authors	Study adequacy						
						Nominal dose (mg/(kg × d))*	Regimen	Duration (months)				Route	Group size†	Dose-response	Daily dose‡	Duration¶	Quality§	Overall adequacy score
										cancer								
Eränkö et al. 1959a	s.c.	Rat	?	M	60	2.5	0.5 mg/rat daily	9	- hyperplasia of adrenal medulla - no adrenal tumors	- several dose adjustments during study due to toxicity - final body weight 200 g as in controls (uninjected) - not designed as a carcinogenicity study	n.a.	-	-	-	+	-	-	1
Eränkö et al. 1959b	s.c.	Guinea pig	?	?	N: 7 C: 4	8	4 mg/animal daily	8.5	- no histopathological effect on adrenals - hyperplastic effects in rats reproduced (sub-study)	- acute toxicity - final body weight 720 g (average estimated to 500 g) - not designed as a carcinogenicity study	n.a.	-	-	-	+	-	-	1
Eränkö et al. 1959b	s.c.	Mouse	?	?	N: 7 C: 5	3.3	0.1 mg/mouse daily	9	- no histopathological effect on adrenals - hyperplastic effects in rats reproduced (sub-study)	- acute toxicity - final body weight 30 g - not designed as a carcinogenicity study	n.a.	-	-	-	+	-	-	1
Thienes 1960	s.c.	Rat	?	?	50	10	up to 5 mg/kg, twice per day	12	- no histopathological effect on adrenals	- not designed as a carcinogenicity study, but may help interpreting Staemmler's results on medulla	n.a.	-	-	-	+	-	-	1
Schuller et al. 1995	s.c.	Hamster	Syrian	M	20	0.2	1 mg/kg NBT, thrice weekly	16	- no tumors by nicotine	- no statistics reported	negative	-	-	-	-	+	-	1
Galitovskiy et al. 2012	s.c.	Mouse	A/J	F	N: 15 C: 5	1.1	5 d/week 3 mg NHT/kg, 5 d/week	24	- no tumors in control group: 0/5 - uterine leiomyosarcomas: 3/14 - quadriceps rhabdomyosarcomas: 8/14 - lung adenoma: 1/14 - overall tumor incidence: 78% vs. 0% in controls	- insufficient statistical power - no statistics performed - less lung tumors than in historical control data - dosing reportedly at LD_{50}, but mortality only 1/15 - dosing "approximates that consumed by a nicotine addict"	positive	-	-	-	++	-	-	2

Supplementary Table 1 (continued)

Reference	Route of adminis-tration	Species	Strain	Sex	Group size: Nic./ Contr.	Nicotine			Nicotine effects	Comments	Conclusion on *per se* carcino-genicity by authors	Study adequacy						
						Nominal dose (mg/(kg × d))*	Regimen	Duration (months)				Route	Group size†	Dose-response	Daily dose‡	Dura-tion¶	Qual-ity§	Overall adequacy score
von Otto 1911	i.v.	Rabbit	?	?	10/3	?	Daily for four months 0.01%, then 0.1% nicotine; unclear volume of administration	10	- no neoplastic effects described	- study was intended for a thorough histopathological examination of the heart - no clear body weight effect due to nicotine exposure	n.a.	-	-	-	-	-	-	0
Kosdoba 1930	i.v.	Rabbit	?	F, M	12/6	approx. 0.4	0.02 to 1.5 mg/d	≤8	- no neoplastic effects described	- macroscopic pathology was performed - study was intended to investigate adrenal effects, but cardiovascular effects were also described - massive body weight decrease due to nicotine	n.a.	-	-	-	-	-	+	1
Schmähl & Habs 1976	i.p.	Rat	Sprague-Dawley	F+M	72	0.3	2 mg/(kg × week)	20	- 4/67 tumor incidence, same as in control	- shorter survival in nicotine group	negative	-	-	-	+	+	-	2

i.p., intraperitoneal; n.a., not available; NBT, nicotine bitartrate; NHT, nicotine hydrogen tartrate; s.c., subcutaneous.
*Assumptions for mice: 25 g body weight and 5 ml of daily water consumption, if no actual data provided.
†Sufficient if approximately 100 or higher.
‡Sufficient if sign of toxicity reported or if estimated to be higher than in human nicotine users (≥1 mg/(kg × d)).
¶Sufficient if ≥18 months.
§Subjective score considering, e.g. availability of biomonitoring data.
||Assuming a daily drinking water volume of 10 ml and a body weight of 100 g.

Supplementary Table 2. Survey of studies relevant to assess the potential of nicotine to modulate carcinogenesis in animals. Section #1 are physical/chemical/transgenic studies, and Section #2 are cancer xenograft studies. Shaded rows indicate studies with low-adequacy scores (≤2); narratives on these low-scoring studies are presented in Supplementary Table 4.

Section #	Reference	Route of adminis-tration	Species	Strain	Sex	Group size: Nic./Contr.	Co-exposure*	Nicotine			Nicotine effects	Comments	Conclusion on modulating activity by authors	Study adequacy					
								Nominal dose (mg/(kg × d))†	Regimen	Duration (months)				Route	Group size‡	Dose-re-sponse	Daily dose¶	Qual-ity§	Overall adequacy score
1	Freedlander et al. 1956	oral	Mouse	?	?	100	c: UV light: 5 months	18	drinking water	7	- macroscopic examination of ear and eye tumors - no change in tumor incidence by nicotine	- only abstract available - increasing nicotine dose during course of study - max. daily dose - no group with nicotine only to assess interaction	negative	+	+	-	+	-	3
1	Liu et al. 2011	oral	Rat	Wistar	F	12	s: MNU: 10 mg/kg, 4 x, every 2 weeks	2.1, 6.4, and 11	intragastric 5 to 25 mg/kg, thrice weekly for 8 weeks	4	- claimed dose-dependent enlargement of MNU-induced bladder tumors - two metastases in high nicotine dose group - dose-dependent increase in p53 mutation	- no actual data presented on tumor growth	positive	+	-	+	+	-	3
1	Murphy et al. 2011	oral	Mouse	A/J	F	18	s: NNK: 80 mg/kg, i.p.	6	drinking water 0.2 NHT mg/ml	11	- no effect on NNK-induced tumor multiplicity - no effect of tumor size or degree of progression - no effect on metabolic activation of NNK and DNA adducts - urinary nicotine: 2170 ng/ml: cotinine: 5700 ng/ml - plasma nicotine: 0.66 ng/ml: cotinine: 31 ng/ml	- water consumption: 15 ml/week - nicotine was given before and after NNK or all over with no difference for NNK-derived tumorigenesis	negative	+	-	-	+	++	4

Supplementary Table 2 (continued)

Section #	Reference	Route of adminis-tration	Species	Strain	Sex	Group size: Nic./Contr.	Co-exposure*	Nicotine			Nicotine effects	Comments	Conclusion on modulating activity by authors	Study adequacy					
								Nominal dose (mg/(kg × d))†	Regimen	Duration (months)				Route	Group size‡	Dose-re-sponse	Daily dose¶	Qual-ity§	Overall adequacy score
1	Maier et al. 2011 (Figure 1)	oral	Mouse	AB6F1 (F1 of A/J and C57Bl6)	?	10	s: NNK: 100 mg/kg, i.p. for 3 weeks	10	drinking water 0.1 mg/ml, racemic mixture	3	- no effect on lung tumor multiplicity w/o NNK (1/30 tumors in nicotine group vs. 0/10 in control - no effect on NNK-induced tumor multiplicity - lack of effect was reproduced after only one NNK administra-tion and larger group size - no effect of tumor volume - serum cotinine: 137 ng/ml	- daily dose corrected to resemble (-)-nicotine - serum cotinine concentration claimed to be comparable to that of patch users	negative	+	-	-	+	++	4
1	Maier et al. 2011 (Figure 3)	oral	Mouse	*Kras*^{LA2}	?	5	mutant K*ras*	10	drinking water 0.1 mg/ml, racemic mixture	0.5, 1.5, and 5	- no effect on tumor multiplicity and burden - no effect on tumor multiplicity and burden - no effect on survival	- exposure to nicotine from age of 0.5 months for 0.5 months and from 1.5 months for 1.5 months or until death (approx. 5 months) - dose estimated for group starting at age 1.5 months - similar cotinine levels as in parallel sub-study with F1 of A/J and C57Bl6 mice - no statement of tumor multiplicity for this part	negative	+	-	-	+	+	3
1	Hermann et al. 2014	oral	Mouse	K-Ras$^{+/LSLG12Vgeo}$	?	7	mutant K*ras*	20	drinking water 0.1 mg/ml	18	- >10-fold increase in area of pancreatic intraepithelial neoplasia lesions - increased grade/severity of lesions - urinary cotinine at 210 ng/ml (no indication when collected), considered similar to level of intermediate smokers	- no effect on body weight - many mechanistic aspects included - drinking water was supplemented with 2% sucrose	positive	+	-	-	+	+	3

Supplementary Table 2 (continued)

Section #	Reference	Route of administration	Species	Strain	Sex	Group size: Nic./Contr.	Co-exposure*	Nicotine			Nicotine effects	Comments	Conclusion on modulating activity by authors	Study adequacy					
								Nominal dose (mg/(kg × d))†	Regimen	Duration (months)				Route	Group size‡	Dose-response	Daily dose¶	Quality§	Overall adequacy score
1	Hermann et al. 2014	oral	Mouse	K-Ras$^{+/LSLG12D}$; Trp53$^{+/LSLR172H}$	?	7	mutant K*ras*	20	drinking water 0.1 mg/ml	20	-4-fold increase in area of pancreatic intraepithelial neoplasia lesions - increased grade/severity of lesions - increased number of circulating pancreatic cells considered indicative of a metastatic phenotype	- no data on body weight and urinary cotinine levels - many mechanistic aspects included - drinking water was supplemented with 2% sucrose	positive	+	-	-	+	+	3
1	Nishikawa et al. 1992	oral	Hamster	Syrian Golden	F	28	s: N-nitrosobis (2-oxopropyl)amine, 10 mg/kg once per week for 3 weeks, s.c.	2.5 ||	25 ppm in drinking water	9	- tendency to enhanced pancreatic carcinogenesis claimed by authors, no statistically significant effects for nicotine - adenocarcinoma incidence increased from 39% without nicotine to 43% with nicotine, dysplasia incidence from 64% to 86% - adenocarcinoma multiplicity increased from 0.4±0.6 to 0.6 ± 0.9, for dysplasia from 2.3 ± 2.2 to 3.1 ± 2.9	- no body weight effect due to nicotine treatment - caffeine effect (tested in parallel to nicotine) seemed to be more pronounced and adenocarcinoma multiplicity was statistically significantly increased - histopathology was well conducted with serial sectioning in four pancreatic lobes	positive	+	-	-	+	+	3
1	Freedlander & French 1956	oral	Mouse	A	?	50	s: urethane: 800 mg/kg, i.p., 2 x	17	drinking water	4	- incidence of pulmonary adenomas not changed by nicotine	- only abstract available - increasing nicotine dose during course of study - max. daily dose - no group with nicotine only to assess interaction	negative	+	-	-	+	-	2
1	Ito et al. 1984	oral	Rat	F344	M	20 to 30	s: BBN: 0.01% and 0.05% in drinking water, 4 weeks	1	dietary 0.0025%	8	- no effect on bladder carcinogenesis - no other organs examined	- significant promoting effects by other compounds tested in this study - assuming 16 g food intake per day and an average body weight of 400 g - mentioning of a nicotine only group, no further information	negative	+	-	-	+	-	2

Supplementary Table 2 (continued)

Section #	Reference	Route of administration	Species	Strain	Sex	Group size: Nic./Contr.	Co-exposure*	Nicotine			Nicotine effects	Comments	Conclusion on modulating activity by authors	Study adequacy					
								Nominal dose (mg/(kg × d))†	Regimen	Duration (months)				Route	Group size‡	Dose-response	Daily dose¶	Quality§	Overall adequacy score
1	Nakada et al. 2012	oral	Mouse	A/J	F	?	s: NNK: 80 mg/kg, i.p.	20	drinking water 0.1 mg/ml	4	- no increase in tumor multiplicity - no adenocarcinoma	-	negative	+	-	-	+	-	2
1	Chen & Squier 1990	cheek pouch	Hamster	Syrian	M	10	c: DMBA: 10 mg/kg	13	60 mg/ml, thrice weekly in 50 µl applications: 3 mg	3	-increased tumor multiplicity in cheek pouches by co-treatment with nicotine - trend to larger size of cheek pouch tumors due to nicotine - no effect by nicotine alone	- average dose estimated using a body weight of 100 g assuming full retention	positive	+	-	-	+	-	2
1	Chen et al. 1994	cheek pouch	Hamster	Syrian	M	6 to 10	c: NNN: 10 mg/ml or c: NNK: 10 mg/ml	13	60 mg/ml, thrice weekly in 50 µl applications: 3 mg	3	- 2/6 hamsters with squamous cell papillomas in forestomach after combined treatment with NNK and nicotine - epithelial changes in cheek and forestomach more pronounced after co-treatment	- average dose estimated using a body weight of 100 g, assuming full retention	positive	+	-	-	+	-	2
1	Bock & Tso 1976 (Table IV)	dermal	Mouse	?	?	96	s: DMBA: 5 mg/kg and c: crude fractions of unburnt tobacco	?	skin painting, 5 to 26 mg/ml	6	- increased tumor incidence (10%) at 12 mg/ml, including 1 malignant tumor	- reduced survival at high nicotine combination - further experimental details missing	positive	+	+	-	-	-	2
1	Bock & Tso 1976 (Table IV)	dermal	Mouse	?	?	96	s: DMBA: 5 mg/kg	?	skin painting, 12 mg/ml	6	- no difference in tumor incidence (1%) vs. control (2%)	- volume of administration unknown for nicotine, thus, dose cannot be calculated	negative	+	+	-	-	-	2
1	Bock & Tso 1976 (Table V)	dermal	Mouse	?	?	48	s: DMBA: 5 mg/kg and c: crude fractions of unburnt tobacco	?	skin painting, 0 to 14 mg/ml	8	- increased tumor incidence (up to 11%), including 1 malignant tumor	- no effect on survival at high nicotine combination	positive	+	-	+	-	-	2
1	Bock & Tso 1976 (Table V)	dermal	Mouse	?	?	48	s: DMBA: 5 mg/kg	?	skin painting, 0 to 14 mg/ml	8	- no difference in tumor incidence (0 to 2%) vs. control (2%)	- further experimental details missing, dose cannot be calculated	negative	+	-	+	-	-	2

Supplementary Table 2 (continued)

Section #	Reference	Route of administration	Species	Strain	Sex	Group size: Nic./Contr.	Co-exposure*	Nicotine			Nicotine effects	Comments	Conclusion on modulating activity by authors	Study adequacy					
								Nominal dose (mg/(kg × d))†	Regimen	Duration (months)				Route	Group size‡	Dose-response	Daily dose¶	Quality§	Overall adequacy score
1	Bock 1980 (Table 1)	dermal	Mouse	?	?	96	s: DMBA: 500 µg/ml c: TPA: 0.5 µg/ml	?	skin painting, 3 mg/ml in 0.2 ml applications	8.5	- no nicotine effect on TPA-mediated promotion - author concluded that nicotine in earlier observed cocarcinogenesis studies acted through mechanisms other than initiation or promotion	- further experimental details missing, dose cannot be calculated	negative	+	-	-	-	-	1
1	Bock 1980 (Table 2)	dermal	Mouse	ICR Swiss	F	C:75 N: 50	c: BaP: 10 µg/ml and TPA: 0.6 µg/ml combined	?	skin painting, 2.5 and 5 mg/ml 10 times/week	9.5	- nicotine was mixed with BaP and TPA - significant cocarcinogenicity at both nicotine dose levels	- further experimental details missing, dose cannot be calculated	positive	+	-	+	-	-	2
1	Bock 1980 (Figure 2)	dermal	Mouse	?	?	?	c: BaP: 10 µg/ml and TPA: 0.6 µg/ml combined	?	skin painting, 0 to 6 mg/ml	up to 8	- nicotine was mixed with BaP and TPA - at moderate dose levels increased carcinogenicity - at high dose level delay in carcinogenicity - author concluded that nicotine acted as cocarcinogen	- further experimental details missing, dose cannot be calculated - reduced survival at high nicotine dose	positive	+	-	+	-	-	2
1	Bock 1980 (Figure 3)	dermal	Mouse	ICR Swiss	F	90	c: BaP: 750 µg/ml c: TPA: 3 µg/ml	?	skin painting, 3 mg/ml with each BaP or TPA	6	- nicotine was added either during initiation with BaP (3 weeks) or during promotion with TPA - no nicotine effect on either initiation or promotion	- further experimental details missing, dose cannot be calculated	negative	+	-	-	-	-	1
1	Rana & Bhagat 1970	s.c.	Mouse (new-born)	?	?	?	s: BaP: 3 × 3 mg/ mouse, s.c.	3	1 mg/kg thrice daily	> 5	- "immunosympathectomized" mice (by immunization with horse antiserum against nerve growth factor) - no effect by nicotine in either immunized or control groups on BaP-induced tumorigenesis	- only abstract available - insufficient information on study details - contradictory to finding in second study?	negative	-	-	-	+	-	1

Supplementary Table 2 (continued)

Section #	Reference	Route of adminis-tration	Species	Strain	Sex	Group size: Nic./Contr.	Co-exposure*	Nicotine			Nicotine effects	Comments	Conclusion on modulating activity by authors	Study adequacy					
								Nominal dose (mg/(kg × d))†	Regimen	Duration (months)				Route	Group size‡	Dose-re-sponse	Daily dose¶	Qual-ity§	Overall adequacy score
1	Bhagat & Rana 1971	s.c.	Mouse	CD-1 (newborn)	?	30	s: BaP: 3 × 3 mg/ mouse	1.5	0.5 mg/kg thrice daily	6	- delayed appearance of tumors and reduced incidence - larger tumors in nicotine group	- little experimental details reported	negative (protective)	-	-	-	+	-	1
1	Gurkalo & Volfson 1982	s.c.	Rat	?	?	20	c: MNNG: 85 mg/l in dr. water for 8 months	0.14	0.5 mg/kg, twice per week (with interrup-tions)	10	- earlier development and doubled incidence of stomach tumors (67% vs. 30%)	- few tumors in other organs - nicotine-related mortality - no control group with nicotine alone	positive	-	-	-	-	-	0
1	Habs & Schmähl 1984	s.c.	Rat	Sprague-Dawley	F	30	s: MNU: 50 mg/kg, s.c.	0.04	0.4 mg/kg NBT twice weekly	0.2 and 3	- nicotine treatment for 1 week before MNU or 3 months after MNU - 100% incidence of mammary tumors due to MNU - no impact on tumor growth kinetics, histology, and volume - no tumors by nicotine alone	- contrasting doses reported (0.4 or 0.5 mg/kg) - no acute toxicity or body weight effects	negative	-	-	-	-	-	0
1	Schuller et al. 1995	s.c.	Hamster	Syrian	M	20	c: 60% oxygen	0.15	1 mg/kg NBT, thrice weekly	16	- no tumors in hyperoxia and nicotine alone groups - 2 nasal adeno-carcinomas, 2 lung adenomas, 2 lung adenocarcinomas, 1 adrenal cortical adenocarcinoma - severe hyperoxic lung tissue damage - tumors positive for neuroendocrine markers	- lifetime study - no statistics reported - different morphological type of lung cancer compared to hyperoxia/N-nitrosamines - nicotine was contaminated with 0.1% nornicotine	positive	-	-	-	-	+	1
1	Bersch et al. 2009	s.c.	Mouse	CF1	M	N: 27 C: 0	c: DMBA: 1 mg/ mouse implanted into pancreas	2	daily	1.5	- 52% incidence of pancreatic adenocarcinoma - no concomitant sham control; historic control: 17% incidence - concomitant smoke inhalation group: 13% incidence	- more pronounced preneoplastic effects claimed but not very obvious	positive	-	-	-	+	-	1

Supplementary Table 2 (continued)

Section #	Reference	Route of administration	Species	Strain	Sex	Group size: Nic./Contr.	Co-exposure*	Nicotine			Nicotine effects	Comments	Conclusion on modulating activity by authors	Study adequacy					
								Nominal dose (mg/(kg × d))†	Regimen	Duration (months)				Route	Group size‡	Dose-response	Daily dose¶	Quality§	Overall adequacy score
1	Hayashi et al. 2014	s.c.	Mouse	Balb/c	M	10 to 13	c in 3 periods: i.p. azoxymethane: 12 mg/kg; 2% DSS	3	3 periods of 7 d during DSS treatment	3	- anti-inflammatory (less colitis) - reduced colon cancer multiplicity - reduced tumor size - no difference for adenocarcinoma, though - less precancerous dysplasia in proximal colon	- azoxymethane/DSS used as an established mouse model to induce colitis-associated colon cancer - reduced tumorigenicity considered due to anti-inflammatory effect (via nAChR and CD4+)	negative (protective)	-	-	-	+	+	2
1	Habs & Schmähl 1976	i.p.	Rat	Sprague-Dawley	F+M	64	ENU: 30 mg/kg p.o. on GD 19 to dams	0.3	2 mg/(kg × week) postnatal	>9	- ENU dose-dependent carcinogenicity in offspring (mostly neurogenic but other malignant tumors, too) - no effect by postnatal nicotine	- no information on nicotine carcinogenesis *per se*	negative	-	-	-	-	-	0
1	Davis et al. 2009 (Figure 3)	i.p.	Mouse	A/J	F	8	s: NNK: 100 mg/kg i.p. for 5 weeks	0.4	1 mg/kg thrice weekly	7	- increased multiplicity of lung tumors - increased size of lung tumors - nicotine-induced changes in gene expression	-	positive	-	-	-	-	-	0
1	Iskandar et al. 2013	i.p.	Mouse	A/J	M	16	s: NNK: 100 mg/kg once, 2 weeks before nicotine administration	0.4	1 mg/kg thrice weekly	2	- increased lung tumor multiplicity and volume beyond NNK effect - increased emphysema (mean linear intercept) in combined treatment group vs. control - mechanistic effects	- nicotine administration started two weeks after NNK injection - no control group with only nicotine - advantage of concomitant tumor and emphysema investigation - two independent experiments claimed but identical data for disease and mechanistic endpoints - trend to decreased survival	positive	-	-	-	-	+	1
1	Berger & Zeller 1988	s.c.?	Rat	BD IX	F	12	- s: MNU-induced mammary carcinogenesis (three i.v. doses of 50 mg/kg)	5	Osmotic minipump (no information on route of administration); saline control	1	- No effect of nicotine alone on MNU-driven tumor development (tumor volume) -	- Relatively constant serum nicotine levels (approx. 25 and 45 ng/ml for the two dose levels, respectively); cotinine levels ranged from 163 to 449 ng/ml and from 218 to 635 ng/ml, respectively	negative	-	-	-	1	1	2

Supplementary Table 2 (continued)

Section #	Reference	Route of administration	Species	Strain	Sex	Group size: Nic./Contr.	Co-exposure*	Nicotine			Nicotine effects	Comments	Conclusion on modulating activity by authors	Study adequacy					
								Nominal dose (mg/(kg × d))†	Regimen	Duration (months)				Route	Group size‡	Dose-response	Daily dose¶	Quality§	Overall adequacy score
1	Berger & Zeller 1988	s.c.?	Rat	BD IX	F	12	- s: MNU-induced mammary carcinogenesis (3 i.v. doses of 50 mg/kg) - c: chemotherapy by 1-(2-chloroethyl)-1-nitroso-3-(2-hydroxyethyl)urea i.p. at 4 dose levels including 0	5	Osmotic minipump (no information on route of administration); saline control	1	- Nicotine supported the chemotherapeutic effect at the highest dose of the chemotherapeutic (less median tumor volume, lower median tumor number)	- Relatively constant serum nicotine levels (approx. 25 and 45 ng/ml for the two dose levels, respectively); cotinine levels ranged from 163 to 449 ng/ml and from 218 to 635 ng/ml, respectively	Negative (protective)	-	-	-	1	1	2
2	Jarzynka et al. 2006	oral	Mouse	? nude, ovariectomized	F	2 × 8	A549 cells, 10^7 cells/ mouse s.c. estradiol pellets, s.c.	40 and 20	drinking water 0.2 mg/ml and 0.1 mg/ml	1	- numerical increase in tumor growth - statistically significant increase (additive) with concomitant estradiol exposure - increased cell proliferation in tumor tissue - trend to increased vascularization in tumor tissue, significant with estradiol co-exposure	- authors declare nicotine effect positive although their statistical evaluation does not show significance - negative effects with nicotine at 0.1 mg/ml drinking water (data not shown)	positive/ negative	+	-	+	+	-	3
2	Shin et al. 2004	oral	Mouse	athymic nude BALB/c	?	10	gastric cancer cell line, implanted in gastric wall	11 and 62	drinking water 0.05 mg/ml and 0.2 mg/ml	3	- larger gastric tumor areas, dose-dependent - increased cell proliferation and microvessel density - activation of growth signal transduction - increased COX-2, VEGF expression in tumors - effects prevented by COX-2 inhibitor	- body weight effect at the high nicotine dose	positive	+	-	+	+	+	4

Supplementary Table 2 (continued)

Section #	Reference	Route of administration	Species	Strain	Sex	Group size: Nic./Contr.	Co-exposure*	Nicotine			Nicotine effects	Comments	Conclusion on modulating activity by authors	Study adequacy					
								Nominal dose (mg/(kg × d))†	Regimen	Duration (months)				Route	Group size‡	Dose-response	Daily dose¶	Quality§	Overall adequacy score
2	Wong et al. 2007	oral	Mouse	nude BALB/c	F	?	colon cancer cell line, s.c.	10 and 40	drinking water 0.05 mg/ml and 0.2 mg/ml	0.8	- increased tumor volume, dose-dependent - increased microvessel density - plasma cotinine: 9, 43, 169 ng/ml for sham, low, high doses - increased plasma adrenaline in high dose group - mechanistic investigations	- no effect on water consumption and body weight - unclear source of cotinine in sham-treated group	positive	+	-	+	+	+	4
2	Maier et al. 2011 (Figures 4 and 5)	oral	Mouse	AB6F1 (F1 of A/J and C57Bl6)	?	5	3 NNK-transformed cell lines, 1×10^5 cells/ mouse, s.c.	10	drinking water 0.1 mg/ml, racemic mixture	0.5	- no effect on tumor growth - no effect on metastasis	- daily dose corrected to resemble (-)-nicotine	negative	+	-	-	+	+	3
2	Li et al. 2015	oral	Mice	Nude BALB/c	F	?	- PC9 NSCLC cells, 5 x 10^6/mouse, s.c., - s/c: erlotinib 100 mg/(kg × d) by gavage between days 21 and 36	20	Drinking water, 0.1 mg/ml	0.5	- Slight but statistically significant increase in tumor growth - Further growth inhibitable by erlotinib (epidermal growth factor receptor antagonist) - Serum cotinine level approx. 37 ng/ml - Serum cotinine level in controls without nicotine 14 to 20 ng/ml - In tumor tissue, nicotine inhibited the antagonistic effect of erlotinib on epidermal growth factor receptor phosphorylation	- Although tumor growth was similar for i.v. and oral nicotine administration, the effect of erlotinib was more pronounced upon continued i.v. than oral nicotine exposure - Nicotine also seemed to inhibit the effect of erlotinib when compared to a control without nicotine - No effect on body weight by nicotine	Positive	+	-	-	+	+	3
2	Pratesi et al. 1996	s.c.	Mouse	athymic nude Swiss	F+M	5 to 10	tumor fragments developed from SCLC cell lines	0.8 and 8	osmotic minipump	0.5	- no difference in time to tumor appearance and growth to target volume, regardless whether given during early or established phases of tumorigenesis	-	negative	+	-	+	+	-	3
2	Hao et al. 2013	s.c.	Mouse	RAG2$^{-/-}$ mice	F	15 to 18	murine melanoma cell line, 1×10^6/ mouse, i.v.	13	osmotic minipump	0.75	- doubling of tumor metastasis volume - inhibitable by crossing in β2-nAChR$^{-/-}$	- nicotine biomonitoring data available	positive	+	-	-	+	+	3

Supplementary Table 2 (continued)

Section #	Reference	Route of administration	Species	Strain	Sex	Group size: Nic./Contr.	Co-exposure*	Nicotine			Nicotine effects	Comments	Conclusion on modulating activity by authors	Study adequacy					
								Nominal dose (mg/(kg × d))†	Regimen	Duration (months)				Route	Group size‡	Dose-response	Daily dose¶	Quality§	Overall adequacy score
2	Berger & Zeller 1988	s.c.?	Rat	BD IX	F	8	- Rat L5222 leukemia cells, 10^5/rat	2.5 and 5	Osmotic minipump (no information on route of administration); saline control	0.5	- No effect of nicotine alone on leukemia development and related survival time	- Relatively constant serum nicotine levels (approx. 25 and 45 ng/ml for the two dose levels, respectively); cotinine levels ranged from 163 to 449 ng/ml and from 218 to 635 ng/ml, respectively	negative	-	-	+	+	+	3
2	Berger & Zeller 1988	s.c.?	Rat	BD IX	F	8	- Rat L5222 leukemia cells, 10^5/rat - c: chemo-therapy by cyclo-phospha-mide i.p. 4 dose levels including 0, 4 h after minipump implanta-tion	2.5 and 5	Osmotic minipump (no information on route of administration); saline control	0.5	- Authors see a tentative inhibition by nicotine of the chemotherapy at the lowest cyclophosphamide dose (p=0.05)	- Relatively constant serum nicotine levels (approx. 25 and 45 ng/ml for the two dose levels, respectively); cotinine levels ranged from 163 to 449 ng/ml and from 218 to 635 ng/ml, respectively	positive	-	-	+	+	+	3
2	Paleari et al. 2008	i.v.	Mouse	NOD/ SCID	F+ M	10 to 12	A549-luc cells, intra-thoracic inoculation	0.6	tail vein, daily injections (bolus?)	0.5	- claimed increase in tumor incidence (100% vs. 60% in controls) - claimed inhibition by α7-nAChR antagonist	- examination of tumor growth via lumi-nescence of marked A549 cells; no quant-itative data shown	positive	+	-	-	-	-	1
2	Li et al. 2015	i.v.	Mice	Nude BALB/c	F	?	- PC9 NSCLC cells, 5 x 10^6/ mouse, s.c. - s/c: erlotinib 100 mg/(kg × d) by gavage between days 21 and 36	0.04	0.06 mg/kg, 5 times/ week	0.5	- Slight but statistically significant increase in tumor growth - Further growth inhibitable by erlotinib (epidermal growth factor receptor antagonist) - Serum cotinine level of 370 and 500 ng/ml 30 min after i.v. injection of nicotine - Serum cotinine level in controls without nicotine 14 to 20 ng/ml - In tumor tissue, nicotine inhibited the antagonistic effect of erlotinib on the epidermal growth factor receptor phosphorylation	- Although tumor growth was similar for i.v. and oral nicotine administration, the effect of erlotinib was more pronounced upon continued i.v. than oral nicotine exposure - Nicotine also seemed to inhibit the effect of erlotinib when compared to a control without nicotine - No effect on body weight by nicotine	positive	-	-	-	-	+	1

Supplementary Table 2 (continued)

Section #	Reference	Route of administration	Species	Strain	Sex	Group size: Nic./Contr.	Co-exposure*	Nicotine			Nicotine effects	Comments	Conclusion on modulating activity by authors	Study adequacy					
								Nominal dose (mg/(kg × d))†	Regimen	Duration (months)				Route	Group size‡	Dose-response	Daily dose¶	Quality§	Overall adequacy score
2	Heeschen et al. 2001	oral	Mouse	C57Bl6	?	?	Lewis carcinoma cells, 10^6/ mouse, s.c.	20	drinking water 0.1 mg/ml	0.5	- accelerated tumor growth - increased tumor tissue vascularity	- pathophysiologically relevant nicotine conc. claimed	positive	+	-	-	+	-	2
2	Heeschen et al. 2001	oral	Mouse	C57Bl6	?	?	Lewis carcinoma cells, 10^6/ mouse implanted into lungs	20	drinking water 0.1 mg/ml	0.25	- accelerated tumor growth - increased tumor tissue vascularity	- pathophysiologically relevant nicotine conc. claimed	positive	+	-	-	+	-	2
2	Natori et al. 2003	oral	Mouse	C57Bl6	M	6	colon cancer cell line, 6×10^7 cells/ mouse, s.c.	20	gavage, daily	0.3	- increased tumor volume (approximately 4-fold) - increased capillary density	- nicotine administration started 5 days prior to inoculation	positive	+	-	-	+	-	2
2	Al-Wadei et al. 2009	oral	Mouse	athymic nude	M	?	pancreatic ductal adeno-carcinoma cell line, 3×10^6 cells/ mouse, s.c.	19	drinking water 0.2 mg/ml NBT	1	- growth of inoculated cells to larger tumors - inhibitable by γ-aminobutyric acid as a cAMP antagonist leading to the assumption of catecholamine-mediated effects	- dose estimated assuming 6.9 ml water consumption/ day - increased levels of adrenal and noradrenaline - no effect on body weight development or acute toxicity - no difference in drinking water uptake	positive	+	-	-	+	-	2
2	Lee et al. 2010 (Figure 4C)	oral	Mouse	Balb/c NOD-SCID	F	5	trans-formed mammary gland cell line, 5×10^6 cells/ mouse, s.c.	2000	drinking water 10 mg/ml	1.2	- human cell line made transgenic for condi-tional overexpression of α9-nAChR - cell transformation upon in vitro nicotine exposure in nAChR-overexpressing cells - transformed cells used for xenograft study - faster tumorigenic growth of nAChR-overexpressing cells - accelerated growth by nicotine exposure regardless of nAChR overexpression	- reported concentration of nicotine in drinking water and thus estimated nicotine uptake per day seemingly impossible - should have seen nicotine toxicity effects, but none reported - study can thus not be evaluated for its nicotine-related effects	positive	+	-	-	?	-	1

Supplementary Table 2 (continued)

Section #	Reference	Route of administration	Species	Strain	Sex	Group size: Nic./Contr.	Co-exposure*	Nicotine			Nicotine effects	Comments	Conclusion on modulating activity by authors	Study adequacy					
								Nominal dose (mg/(kg × d))†	Regimen	Duration (months)				Route	Group size‡	Dose-response	Daily dose¶	Quality§	Overall adequacy score
2	Lee et al. 2010 (Figure 3C)	oral	Mouse	Balb/c NOD-SCID	F	5	mammary gland adeno-carcinoma cell line, 5×10^6 cells/ mouse, s.c.	2000	drinking water 10 mg/ml	1.5	- human cell lines with or without stably expressed short interfering RNA against α9-nAChR - inhibited tumor growth by interference with nAChR - trend to increased tumor growth with nicotine in controls but not upon interference with nAChR	- reported concentration of nicotine in drinking water and thus estimated nicotine uptake per day seemingly impossible - should have seen nicotine toxicity effects, but none reported - study can thus not be evaluated for its nicotine-related effects	positive	+	-	-	?	-	1
2	Al-Wadei et al. 2012	oral	Mouse	athymic nude	M	10	2 NSCLC cell lines, 3×10^6/ mouse, s.c.	14	drinking water 0.2 mg/ml NBT	1	- growth of inoculated cells to larger tumors - inhibitable by concomitant GABA administration - inhibitable by GABA as a cAMP antagonist leading to the assumption of catecholamine-mediated effects	- conflicting information on nicotine concentration in drinking water: Figure 1: 200 mg/ml; Methods: 1 µMol/l - In analogy to the previous study with similar design, the same nicotine concentration was assumed for this study.	positive	+	-	-	+	-	2
2	Nakada et al. 2012	oral	Mouse	C57Bl6	F	?	Lewis carcinoma cells, 1×10^6 cells/ mouse, s.c.	20	drinking water 0.1 mg/ml	0.5	- increased tumor volume	-	positive	+	-	-	+	-	2
2	Banerjee et al. 2013	oral	Mouse	athymic nude	M	10	pancreatic ductal adeno-carcinoma cell line, 3×10^6/ mouse, s.c.	0.011	drinking water 0.16 µg/ml	1	- no effect on tumor volume - reduced therapeutic response to gemcitabine	- dose estimated by authors probably expressed as NBT - dose low by intention to avoid cancer cell proliferation and to study drug-induced apoptotic effect on cancer cells	negative	+	-	-	-	+	2
2	Khalil et al. 2013	oral	Mouse	nude	?	3	human glioma cells GBM12, intracranial	1000	drinking water, 5 mg/ml	0.5	- increased tumor growth	- GBM cells were labeled and tumors were screened with luciferase activity - unbelievably high nicotine dose - no statistics	positive	-	-	-	+	-	1

Supplementary Table 2 (continued)

Section #	Reference	Route of administration	Species	Strain	Sex	Group size: Nic./Contr.	Co-exposure*	Nicotine			Nicotine effects	Comments	Conclusion on modulating activity by authors	Study adequacy					
								Nominal dose (mg/(kg × d))†	Regimen	Duration (months)				Route	Group size‡	Dose-response	Daily dose¶	Quality§	Overall adequacy score
2	Banerjee et al. 2014	oral	Mouse	athymic nude	M	10	pancreatic ductal adeno-carcinoma cell line, 3×10^6/ mouse, s.c.	0.016	drinking water 0.16 µg/ml NHT	1	- no effect on tumor volume - reduced therapeutic response to gemcitabine, counteracted by concomitant GABA administration	- dose low by intention to avoid cancer cell proliferation and to study drug-induced apoptotic effect on cancer cells - no nicotine-only group	negative	+	-	-	-	-	1
2	Liu et al. 2015	oral	Mice	Nude BALB/c	M	6	- A549 cells, 5 x 10^6/mouse s.c. - c: chemo-prevention studied with an extract of Nelumbo nucifera Gaertn, 50 mg/kg i.p. thrice/ week	0.03	Drinking water, 1 µM	0.7	- increased tumor weight - nucifera extract partly inhibited tumor growth but nicotine effect was still apparent - mechanistically, nicotine enhanced β-catenin expression and decreased Bax expression and apoptosis in the tumor tissue	- the nicotine dosing appeared to be slightly toxic to the mice, because there was an increase in serum glutamic-pyruvate transaminase	positive	+	-	-	-	-	1
2	Davis et al. 2009	dermal	Mouse	BALB/c	F	?	mouse adeno-carcinoma cells, s.c.	25	patch	0.3	- increased tumor size derived from implanted cells - urinary cotinine: 5000 ng/ml	- nicotine dose estimated by authors based on content and size of patches - contrasting group sizes reported (8 or 14 or 16)	positive	+	-	-	+	-	2
2	Warren et al. 2012	s.c.	Mouse	athymic nude Foxn1	M	?	human lung cancer cells, 1.5×10^6/ mouse, s.c.	0.9	60 µg/ mouse, every 2nd day	1	- no effect on xenograft growth - accelerated growth after therapy, if nicotine was present during or shortly after radiotherapy or chemotherapy combined with radiotherapy	- apparently first report to show nicotine's resistance to cancer therapy *in vivo* - maximally tolerated dose - body weight approximately 34 g at end of study	negative	-	-	-	-	+	1
2	Davis et al. 2009 (Figure 1)	i.p.	Mouse	BALB/c	F	8	mouse adeno-carcinoma cells, 1×10^6 cells/ mouse, s.c.	0.4	1 mg/kg thrice weekly for 2 weeks	0.5	- increased tumor volume derived from implanted cells - urinary cotinine: 3000 ng/ml	- unclear but very small group sizes - lower cotinine levels in urine than after patch administration, yet more pronounced effect on tumor growth - urinary cotinine levels similar to human smokers	positive	-	-	-	-	-	0

Supplementary Table 2 (continued)

Section #	Reference	Route of administration	Species	Strain	Sex	Group size: Nic./Contr.	Co-exposure*	Nicotine			Nicotine effects	Comments	Conclusion on modulating activity by authors	Study adequacy					
								Nominal dose (mg/(kg × d))†	Regimen	Duration (months)				Route	Group size‡	Dose-response	Daily dose¶	Quality§	Overall adequacy score
2	Davis et al. 2009 (Figure 2)	i.p.	Mouse	BALB/c	F	16	mouse adeno-carcinoma cells, 1×10^6 cells/ mouse, s.c.	0.4	1 mg/kg thrice weekly for 3 weeks	1	- dorsal tumors removed, nicotine exposure for 2 more weeks - higher tumor recurrence after surgical removal - increased pulmonary metastasis from dorsal tumors	-	positive	-	-	-	-	-	0
2	Maier et al. 2011 (Figure 5)	i.p.	Mouse	F1 of A/J and C57Bl6	?	?	2 NNK-trans-formed cell lines, s.c.	?	0.4 mg/kg daily?, racemic mixture	0.5	- no effect on tumor growth	- daily dose corrected to resemble (-)-nicotine - acute nicotine toxicity observed - unknown frequency of injections	negative	-	-	-	-	-	0
2	Molfino et al. 2011	i.p.	Rat	Fischer	M	8	MCA sarcoma cells, s.c. into flank, 10^6 cells per rat	-	200 mg/kg NHT, two times for 3 consecutive days	0.6	- no effect on tumor weight - reduced serum IL-1 levels, no effect on IL-6	- little but protective effect on body weight decline (tumor-associated anorexia-cachexia syndrome) - huge nicotine dose	Negative	-	-	-	+	+	2
2	Treviño et al. 2012	i.p.	Mouse	athymic nude SCID	F	4 ?	pancreatic ductal cancer cells, sub-capsular pancreatic injection	0.4	1 mg/kg thrice weekly	1	- tumor volume doubled - more tumor metastasis to liver - mechanistic investigations	- major tumor results reproduced within study	positive	-	-	-	-	-	0
2	Pillai et al. 2015	i.p.	Mouse	SCID-beige	?	6	- A549 cells expressing luciferase and +/- β-arrestin-1-specific shRNA, orthotopically implanted into left lung	?	?, every other day	1.7	- sh-control cells grew to larger tumors (more luciferase fluorescence) upon nicotine exposure - sh-control cell-treated mice displayed metastases to brain, adrenal glands, and liver (probably not statistically significantly different to control without nicotine) - Mice implanted with β-arrestin-1 knocked-out A549 cells did not respond to nicotine with tumor growth or metastasis - Nicotine also seemed to enhance lung tissue staining for vimentin	- no information on nicotine dose - mechanistic study to demonstrate the role of β-arrestin-1 in mediating nicotine-induced effects	positive	-	-	-	-	-	0

Supplementary Table 2 (continued)

Section #	Reference	Route of adminis-tration	Species	Strain	Sex	Group size: Nic./Contr.	Co-exposure*	Nicotine			Nicotine effects	Comments	Conclusion on modulating activity by authors	Study adequacy					
								Nominal dose (mg/(kg × d))†	Regimen	Duration (months)				Route	Group size‡	Dose-re-sponse	Daily dose¶	Qual-ity§	Overall adequacy score
											and fibronectin indicative of epithelial-to-mesenchymal transition								
2	Yuge et al. 2015	i.p.	Mouse	Nude athymic BALB/c	?	10	- human bladder cancer cell line T24, 2 x 10^6, s.c.; c: PI3K/mTOR dual inhibitor NVP-BEZ235 by daily gavage: c: cis-platin, single i.p. dose at beginning, 5 mg/kg	0.43	1 mg/kg, thrice per week	0.6	- increased tumor volume vs. vehicle control - nicotine prevented chemopreventive effect (reduced tumor growth) by cis-platin	· drug administration was only started when a certain tumor volume had been achieved · accompanied by in vitro and immunohistochemical mechanistic studies · tumor growth without or with nicotine exposure was both inhibited by NVP-BEZ235 · the latter inhibitory effect was not affected by concomitant cis-platin treatment in nicotine-exposed mice	positive	-	-	-	+	-	1
2	Improgo et al. 2013	?	Mouse	athymic nude	?	?	DMS-53 cells, s.c. into hind flank	0.6	24 mg/kg daily by osmotic minipump	1	- approx. 4-fold increase in tumor volume	- additional mechanistic data	positive	-	-	-	-	-	0

BaP, benzo[*a*]pyrene; BBN, N-butyl-N-(4-hydroxybutyl)nitrosamine; BW, body weight; DMBA, dimethyl-benz[*a*]anthracene; DSS, dextrane sulfate sodium; ENU, N-ethyl-nitrosurea; GABA, γ-aminobutyric acid; GD, gestational day; i.p., intraperitoneal; i.v., intravenous; LTDL, Legacy Tobacco Documents Library; MNNG, N-methyl-*N'*-nitro-N-nitrosoguanidine; MNU, N-methyl-nitrosurea; NBT, nicotine bitartrate; NHT, nicotine hydrogen tartrate; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*-nitrosonornicotine; NSCLC, non-small cell lung cancer; p.o., *per os*; s.c., subcutaneous; SCLC, small cell lung cancer; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.
*In case of chemical co-exposures: c: concomitant with nicotine; s: sequential during and after nicotine exposure.
†Assumptions for mice: 25 g body weight and 5 ml of daily water consumption, if no actual data provided.
‡Sufficient if approximately 100 or higher.
¶Sufficient if sign of toxicity reported or if estimated to be higher than in human nicotine users (≥1 mg/(kg × d).
§Subjective score considering, e.g. availability of biomonitoring data.
||Assuming a daily drinking water volume of 10 ml and a body weight of 100 g.

Supplementary Table 3. Survey of studies relevant to assess the potential of nicotine metabolites to induce or modulate carcinogenesis in animals. Shaded rows indicate studies with low-adequacy scores (≤2); narratives on these low-scoring studies are presented in Supplementary Table 4.

Reference	Route of administration	Species	Strain	Sex	Group size: .../Contr.	Co-exposure	Metabolite				Results	Comments	Conclusion on modulating activity by authors	Study adequacy						
							Type	Nominal dose (mg/(kg × d))	Regimen	Duration (months)				Route	Group size*	Dose-response	Daily dose†	Duration‡	Quality¶	Overall adequacy score
Truhaut et al. 1964	oral	Rat	Wistar	F+M	Cot: ≥60 C: ≥15	-	cotinine	63	drinking water 0.5 mg/ml	18	- 12/15 rats that died had malignant lesions vs. no tumors in 15 corresponding controls - most often lymphosarcomas located particularly in the large intestine - several benign lesions at terminal sacrifice	- dose estimated assuming water consumption of 50 ml/d and body weight of 400 g - toxicity, mainly during first 6 months	positive	+	-	-	+	+	-	3
LaVoie et al. 1985	oral	Rat	F344	M	33	-	cotinine	45	drinking water 1 mg/ml	18	- not carcinogenic, regardless of site (42 tumors vs. 39 tumors in control)	- decreased body weight development - dose estimated based on an average water consumption of 12 ml/d and a BW of 400 g	negative	+	-	-	+	+	+	4
LaVoie et al. 1985	oral	Rat	F344	M	NNO: 39 C: 33	-	*trans*-NNO	10	drinking water 0.2 mg/ml	18	- not carcinogenic, regardless of site (28 tumors vs. 39 tumors in control)	- decreased body weight development - dose estimated based on an average water consumption of 18 ml/d and a BW of 350 g	negative	+	-	-	+	+	+	4
LaVoie et al. 1985	oral	Rat	F344	M	NNO: 39 C: 33	-	*rac*-NNO	10	drinking water 0.2 mg/ml	18	- not carcinogenic, regardless of site (33 tumors vs. 39 tumors in control)	- decreased body weight development - dose estimated based on an average water consumption of 18 ml/d and a BW of 350 g	negative	+	-	-	+	+	+	4
LaVoie et al. 1985	oral	Rat	F344	M	33	s: FANFT	cotinine	45	drinking water 1 mg/ml	18	- no effect on bladder, tongue, palate or fore-stomach tumors induced by FANFT (42 tumors vs. 39 tumors in control)	- decreased body weight development - dose estimated based on an average water consumption of 12 ml/d and a BW of 400 g	negative	+	-	-	+	+	+	4

Supplementary Table 3 (continued)

Reference	Route of administration	Species	Strain	Sex	Group size: .../Contr.	Co-exposure	Metabolite				Results	Comments	Conclusion on modulating activity by authors	Study adequacy						
							Type	Nominal dose (mg/(kg × d))	Regimen	Duration (months)				Route	Group size*	Dose-response	Daily dose†	Duration‡	Quality¶	Overall adequacy score
LaVoie et al. 1985	oral	Rat	F344	M	NNO: 39 C: 33	s: FANFT	*trans*-NNO	10	drinking water 0.2 mg/ml	18	- no effect on bladder, tongue, or palate tumors induced by FANFT - increased incidence of FANFT-induced forestomach tumors	- decreased body weight development - dose estimated based on an average water consumption of 18 ml/d and a BW of 350 g	positive	+	-	-	+	+	+	4
LaVoie et al. 1985	oral	Rat	F344	M	NNO: 39 C: 33	s: FANFT	*cis/trans*-NNO	10	drinking water 0.2 mg/ml	18	- no effect on bladder, tongue, or palate tumors induced by FANFT - increased incidence of FANFT-induced forestomach tumors - decreased incidence of FANFT-induced bladder tumors	- decreased body weight development - dose estimated based on an average water consumption of 18 ml/d and a BW of 350 g	positive	+	-	-	+	+	+	4
Freedlander et al. 1956	oral	Mouse	?	?	100	c: UV light: 5 months	NNO	56	drinking water	7	- macroscopic examination of ear and eye tumors - no change in tumor incidence	- only abstract available - max. doses estimated using a body weight of 25 g	negative	+	+	-	+	-	-	3
Nakada et al. 2012	oral	Mouse	A/J	F	?	s: NNK: 80 mg/kg i.p.	cotinine	20 60	drinking water 0.1 and 0.3 mg/ml	4	- increased adenoma multiplicity (significant in high dose group: 4.0 vs. 2.3 in NNK-only group) - no adenocarcinoma	- daily cotinine dose estimated assuming a daily water consumption of 5 ml and 25 g body weight	positive	+	-	+	+	-	-	3
Bock 1980 (Table 2)	dermal	Mouse	ICR Swiss	F	C: 75 Cot: 45	c: BaP: 10 µg/ml and TPA: 0.6 µg/ml combined	cotinine	?	2.5 and 10 mg/ml 10 times/ week	9.5	- cotinine was mixed with BaP and TPA - cocarcinogenicity also observed with cotinine 5 mg/ml, however, the conversion of nicotine was not considered to be responsible for nicotine's observed cocarcinogenicity	- further experimental details missing, dose cannot be calculated - controversial information on high concentration (5 or 10 m) - no information on toxicity	positive	+	-	+	-	+	-	3

Supplementary Table 3 (continued)

Reference	Route of administration	Species	Strain	Sex	Group size: .../Contr.	Co-exposure	Metabolite				Results	Comments	Conclusion on modulating activity by authors	Study adequacy						
							Type	Nominal dose (mg/(kg × d))	Regimen	Duration (months)				Route	Group size*	Dose-response	Daily dose†	Duration‡	Quality¶	Overall adequacy score
Bock 1980 (Table 2)	dermal	Mouse	ICR Swiss	F	C: 75 NNO: 45	c: BaP: 10 µg/ml and TPA: 0.6 µg/ml combined	NNO	?	2.5 and 10 mg/ml 10 times/ week	9.5	- NNO was mixed with BaP and TPA - inhibition of tumorigenicity by NNO - nicotine metabolites might be involved in dose-dependent paradoxical findings with nicotine	- further experimental details missing, dose cannot be calculated - controversial information on high concentration (5 or 10 m) - no information on toxicity	negative (protective)	+	-	+	-	+	-	3
Freedlander & French 1956	oral	Mouse	A	?	50	s: urethane 800 mg/kg, i.p., 2 x	NNO	56	drinking water up to 1.4 mg/ mouse	4	- incidence of pulmonary adenomas not changed by NNO	- only abstract available - increasing NNO dose during course of study - max. doses estimated using a body weight of 25 g - no group with NNO only to assess interaction	negative	+	-	-	+	-	-	2
Schmähl & Osswald 1968	oral	Rat	Wistar	F+M	60	-	cotinine	30	drinking water 0.5 mg/ml	21	- no significant difference to (historic) control rats - 1/60 malignant liver tumor in exposed rats	- no information on toxicity - presumable no concomitant control group	negative	+	-	-	-	+	-	2
Nakada et al. 2012	oral	Mouse	C57Bl6	F	?	Lewis carcinoma cells 10^6/mouse s.c.	cotinine	20	drinking water 0.1 mg/ml	0.5	- increased tumor volume	- daily cotinine dose estimated assuming a daily water consumption of 5 ml and 25 g body weight	positive	+	-	-	+	-	-	2
Boyland 1968	bladder pellet implantation	Mouse	?	F	?	-	cotinine	?	?	?	- 11/69 adenomas mentioned as relevant for possible tumorigenic action by cotinine - no further discussion of the listed carcinomas in this sub-study	- no further experimental details, no concurrent control	positive	-	-	-	-	-	-	0

Supplementary Table 3 (continued)

Reference	Route of administration	Species	Strain	Sex	Group size: .../Contr.	Co-exposure	Metabolite				Results	Comments	Conclusion on modulating activity by authors	Study adequacy						
							Type	Nominal dose (mg/(kg × d))	Regimen	Duration (months)				Route	Group size*	Dose-response	Daily dose†	Duration‡	Quality¶	Overall adequacy score
Boyland 1968	dermal	Mouse	?	M	?	-	cotinine	?	?	?	- no tumors in 39 survivors	- no further experimental details, no concurrent control	negative	-	-	-	-	-	-	0
Boyland 1968	s.c.	Mouse	?	M	?	-	cotinine	?	?	?	- no tumors in 18 survivors	- no further experimental details, no concurrent control	negative	-	-	-	-	-	-	0
Boyland 1968	sub-scapular injection	Mouse (neo-natal)	?	F+M	?	-	cotinine	?	?	?	- two sub-studies with tumors, but not mentioned as relevant	- no further experimental details, no concurrent control	negative	-	-	-	-	-	-	0

BaP, benzo[*a*]pyrene; BW, body weight; FANFT; *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNO, nicotine-*N'*-oxide; s.c., subcutaneous; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.
*Sufficient if approximately 100 or higher.
†Sufficient if sign of toxicity reported or if estimated to be higher than 1 mg/(kg × d).
‡Sufficient if ≥18 months.
¶Subjective score considering, e.g. availability of biomonitoring data.

Supplementary Table 4. Narrative for studies or sub-studies with low-adequacy scoring (with an overall adequacy score ≤2). Section #1 are studies on the potential of nicotine to cause cancer in animals. Studies on the potential of nicotine to modulate cancer are listed in Section #2 (cancer induction by physical/chemical/transgenic means) and Section #3 (cancer xenograft studies). Section #4 are studies with nicotine metabolites.

Section #	Route of administration	Narrative
1	intra-tracheal instillation	Yokohira and colleagues instilled Fischer rats with a nicotine saline solution at doses of 0.05, 0.1, and 0.2 mg/rat, corresponding to approximately 0.15, 0.3, and 0.6 mg/kg, three to nine times over a period of 30 weeks (Yokohira et al. 2012). Tumors would not have been expected in this chronic study, and there were no proliferative changes either in lungs, liver, and kidneys. Fibrosis and marked inflammatory changes were observed, such as neutrophil accumulation and edema, although without dose-dependency. Repeated intratracheal bolus administration with large interim periods does not seem to be quite representative of daily inhalation exposures. In addition, the study suffered from low group sizes (n=3-5), resulting in a low-adequacy score.
1	oral	Schoental and Head (1953) reported in an abstract that CBA mice orally exposed to nicotine for at least 24 months did not develop an increased incidence of lung tumors. No further information on experimental details, such as doses, the extent of necropsy performed, or statistical power, is available for this study, resulting in a low-adequacy score. Truhaut and De Clercq (1961) exposed Wistar rats to nicotine via the drinking water for 24 months. The nicotine concentration and thus the dose were not reported, but the nicotine was administered at a high enough level to cause palatability issues. Only one tumor located in the liver and intestines was observed in the nicotine-exposed group, and no tumors were found in the controls. Many experimental details are unclear, such as the extent of histopathological examinations. In parallel studies, nicotine pyrolysates generated at a temperature of 700°C were administered via the drinking water or s.c. Intestinal tumors at an incidence of 7% were found after administration of the pyrolysates via the drinking water. Due to the lack of study details, e.g. no dosing information, this study has a low-adequacy score.
1	dermal	Schoental and Head (1953) also reported that the dermal application of nicotine did not have any effect on carcinogenicity. The same limits, in terms of lack of experimental detail, and the same adequacy score that applied in the oral administration study also apply to this dermal application study.
1	s.c.	Staemmler (1935) reported about hyperplasia and adenoma development in the adrenal medulla of rats after s.c. nicotine administration for at least 19 months. After daily nicotine doses of 0.5 to 1 mg/kg, acute convulsions were observed. Nicotine doses were apparently adapted from time to time and for particular rats in the study. Tumorigenic changes were observed in the medulla of 40% of the nicotine-treated rats (total group size of 38, unknown control group size), including two rats with adenomas that were observed after 15 and 17 months, respectively. Those rats diagnosed with the adenomas had received daily doses of approximately 0.3 mg/kg, while others had received doses up to 5 mg/(kg × d) but did not progress to actual tumor development. Thus, the degree of progression of the medullar changes does not seem to be dose-dependent in this study. In control rats, morphologic changes of the medulla were only observed very rarely (Staemmler 1936). The author suggested that the increased medullar growth may be related to an increased synthesis of adrenalin due to stimulation by nicotine. However, it should be considered that tumors of the adrenal medulla (pheochromocytomas) occur spontaneously in many rat strains (Greim et al. 2009). Yun and Kim administered nicotine by s.c. injection to Guinea pigs for 6 months (Yun & Kim 1938). Few details were reported. No effect by nicotine was observed, in particular to the adrenal gland, which was the subject of other early studies as well (Staemmler 1935; Thienes 1960). Hueper (1943) exposed rats to s.c. injections of nicotine with increasing doses from 0.2 up to 10 mg per application, which translates to a maximum of approximately 33 mg/(kg × d) assuming a body weight of 300 g. This dose was just below the LD_{50} reported elsewhere for s.c. administration of nicotine to rats (Holmstedt 1988). The study was terminated after 8 months, and no nicotine-related carcinogenicity was reported. The rats showed acute spastic convulsions within several minutes after nicotine administration. In addition, severe morphological changes were observed in various organs and tissues. Congestion of the adrenal medulla, liver, and spleen was often observed, and the testes showed degenerative lesions. The lungs were also often congested and contained more or less extensive hemorrhages; bronchitis and bronchopneumonia were also observed. In parallel groups, ascorbic acid, epinephrine, desoxycorticosterone acetate, or acetylbetamethylcholine chloride were administered in addition to nicotine. These co-treatments modified the chronic toxicity of nicotine to some extent, but again, no tumors were reported. Although this was a chronic study with apparently high nicotine dosing, the observation period for the development of tumors might still have been too short. The observed congestions might be related to the technical performance of the study rather than any toxic effects during the study period. In addition, the observed bronchitis and bronchopneumonia

Supplementary Table 4 (continued)

Section #	Route of administration	Narrative
		may be an indication of infections. These limitations may further complicate the interpretation of this study. Eränkö and colleagues also exposed rats s.c. to nicotine at an average daily dose of 2.5 mg/(kg × d) (Eränkö et al. 1959a). After 9 months, they observed a hyperplasia of the adrenal medulla, but no adrenal tumors. Several dose adjustments during the study were necessary due to nicotine toxicity; however, no body weight effect was seen. A similar study was performed in mice by Eränkö and colleagues, but the study included very small group sizes (Eränkö et al. 1959b). An average s.c. nicotine dose of 3.3 mg/(kg × d) was achieved, which seemed to be toxic. No histopathological effect on the adrenals of these mice was found, but in a sub-study, the effect in rats reported from the previous study could be reproduced. In a sub-study with Guinea pigs, a nicotine effect on adrenals could not be observed either. Thienes (1960) attempted to re-investigate these and other chronic effects attributed to nicotine in earlier studies: rats were exposed s.c. to nicotine for up to 12 months at the higher dose of 10 mg/(kg × d). The nicotine treatment resulted in slightly reduced weight gain of the rats, but there was no increase in size of endocrine organs including the adrenals. Schuller and colleagues administered nicotine s.c. to Syrian golden hamsters in a lifetime study that lasted 16 months (Schuller et al. 1995). The hamsters were injected with nicotine tartrate at 1 mg/kg thrice per week. This corresponds to an average nicotine exposure of 0.2 mg/(kg × d). No signs of nicotine toxicity were reported. No effect on lung morphology was seen. No tumors were reported. The authors concluded that their "findings do not suggest that nicotine is a carcinogen." Galitovskiy and colleagues administered nicotine s.c. to A/J mice at 3 mg nicotine hydrogen tartrate (NHT)/kg for 5 d/week and 24 months (Galitovskiy et al. 2012). On an weekly average, this equals a daily dose of 1.1 mg/kg of nicotine. The authors suggested that they were dosing at the LD_{50} of the test material, however, there was only 1 of 15 nicotine-exposed mice that died. Neither body weights nor any observations regarding nicotine toxicity were reported. Tumors were observed in 78% of the nicotine-exposed mice vs. 0% in the control group, which only contained 5 mice. Three uterine leiomyosarcomas and eight quadriceptal rhabdomyosarcomas were reported. A solitary pulmonary adenoma also occurred in a nicotine-treated mouse. No statistical tests were performed. The authors state that they demonstrated "for the first time that chronic nicotine treatment can induce the development of muscle sarcomas" and that their results suggest to " add nicotine to the list of potential carcinogens in tobacco products and raise concern about the safety of long-term usage of nicotine replacement products." However, rhabdomyosarcomas at the hind legs and lower back were described to be rather frequent spontaneous tumors in A/J mice (34% incidence, Landau et al. 1998). In a chronic mainstream smoke inhalation study in A/J mice, rhabdomyosarcoma incidences of 27% and 43% in female and male control mice were observed, respectively, which tended to decrease with increasing mainstream smoke and thus nicotine exposure concentrations (Stinn et al. 2013). Given the low statistical power of the s.c. nicotine administration study, the definitive conclusions by the authors regarding these rhabdomyosarcomas require confirmation by others. The 7% incidence of lung tumors after 24 months observed in this study is surprisingly low, as most other studies in A/J mice showed 100% incidence at this age (e.g. Stoner & Shimkin 1982).
1	i.v.	Von Otto studied the macroscopic and microscopic effects of a 10-month i.v. nicotine administration in rabbits (von Otto 1911). The daily nicotine administrations were not described in a way that would allow the calculation of doses; however, no body weight effects were described as in another chronic rabbit study with i.v. nicotine administration (Kosdoba 1930). The heart and aorta were carefully examined. No findings related to neoplastic effects were described, but it is unclear whether a full gross pathology examination was indeed performed upon necropsy. Kosdoba intended to investigate the effects of nicotine on the adrenal glands in rabbits (Kosdoba 1930). Nicotine was administered i.v. for up to 8 months at average estimated daily doses of 0.4 mg/kg, which led to a drastic decrement in body weights indicative of the toxicity of the nicotine dose applied. The adrenals were several fold heavier in nicotine-treated compared to control rabbits, which went along with morphological changes mainly in the medulla but also in the cortex. This adrenal hypertrophy was considered indicative of an increased production of adrenalin. Although it seems that a thorough macroscopic pathological examination was performed, neoplastic effects were described neither for the adrenals nor for other organs and tissues.
1	i.p.	Schmähl and Habs (1976) reported on a 20-month study in Sprague-Dawley rats given i.p. injections of nicotine at a dose of 2 mg/(kg × week), which translates to a daily dose of 0.3 mg/kg. The survival in the nicotine-treated rats was about 10% shorter than in the control group. No nicotine effect on tumor incidences (mainly mammary tumors) was observed (7% vs. 6% in nicotine vs. control groups, respectively) in this low-adequacy scoring study.

Supplementary Table 4 (continued)

Section #	Route of administration	Narrative
2	oral	Freedlander and French (1956) investigated the potential interaction of nicotine on urethane-induced lung tumorigenicity in strain A mice. Nicotine was administered via the drinking water at doses increasing from 7 to 17 mg/(kg × d). After 4 months, the incidence of pulmonary adenomas in the nicotine-exposed mice was reportedly similar to that observed in those only treated with urethane. Ito and colleagues tested a series of drugs, food additives, and natural products for promoting activity in a rat model of urinary bladder carcinogenesis, which was initiated with N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) (Ito et al. 1984). Nicotine was administered in the laboratory rodent chow at estimated daily doses of 1 mg/(kg × d). While some of the test compounds promoted bladder carcinogenesis in this model, no effect was observed after nicotine administration. Nakada and colleagues also used 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) initiation in A/J mice and investigated the effect of nicotine via the drinking water at an estimated dose of 20 mg/(kg × d) on tumor growth (Nakada et al. 2012). While there was a weakly positive finding in their Lewis carcinoma model, there was no significant increase in the NNK-initiated model. It should be noted that the drinking water solutions were fortified with saccharine. No information on group size was given.
2	cheek pouch	Chen and Squier (1990) administered nicotine dissolved in sesame oil for three months to the cheek pouches of Syrian golden hamsters, either alone or in combination with dimethyl-benz[*a*]anthracene (DMBA). Assuming full retention and a body weight of 100 g, the daily dose averaged over three applications per week is 13 mg/(kg × d). The cheek pouches from hamsters only treated with nicotine appeared normal. Nicotine in combination with DMBA resulted in significantly more and larger tumors than DMBA alone. Also, a greater degree of dysplasia was observed in the tumors co-treated with nicotine and DMBA. The authors concluded that the concomitant administration of nicotine acted as a "cofactor in DMBA tumorigenesis." Chen and colleagues investigated the potential effect of a co-treatment of nicotine with *N*-nitrosonornicotine (NNN) and NNK using the same model as described above (Chen et al. 1994). In contrast to the previous study, nicotine treatment alone resulted in cheek pouch epithelium hyperplasia, mostly in combination with hyperkeratosis. There were slightly more frequent histologic changes in the cheek pouch when nicotine was combined with NNN than after treatment with either compound alone, but no tumors were seen. In addition, nicotine induced hyperplasia combined with hyperkeratosis in the forestomach of 2/10 of these hamsters. The epithelial changes in the forestomach were more pronounced after co-treatment with nicotine and NNK compared to that with either compound alone, including squamous cell papillomas in 2 of 6 hamsters. The authors concluded "that in mucosal tissues nicotine may enhance the effect of weak carcinogens" such as these tobacco specific nitrosamines.
2	dermal	Bock and Tso (1976) were looking for the responsible component that would account for a tumor promoting activity in unburnt tobacco when applied to the mouse skin painting model for dermal carcinogenesis. Fractionation experiments suggested the involvement of a water-soluble base. To test whether nicotine would play a role, nicotine was added to crude fractions of tobacco at several levels, and this mixture was used as promoter in a two-stage skin painting study with DMBA as the initiator. The tumor promoting activity of the crude tobacco fraction was enhanced by the addition of nicotine up to a certain level and inhibited at higher levels. Nicotine without the tobacco fraction was inactive as a promoter under the conditions of such experiments. Bock (1980) investigated the role of nicotine in dermal carcinogenesis, as this model had commonly been used as a carcinogenicity model for cigarette smoke condensate. A mixture of benzo[a]pyrene (BaP) and 12-*O*-tetradecanoylphorbol-13-acetate (TPA) was administered to mouse skin, and this mixture was fortified with up to 6 mg/ml of nicotine. After 5 months, an overall increased tumor probability due to nicotine admixture was observed. This increase was reduced at the highest nicotine concentration as compared to the lower concentrations. After longer exposures of up to 8 months, a constantly high tumor probability was observed for low and high nicotine concentrations. Thus, nicotine tended to be inhibitory at certain experimental conditions, maybe due to cytotoxicity, as this effect was only obtained at concentrations that were high enough to also cause some mortality in this experiment. Bock conducted further experiments to investigate whether nicotine would act to enhance initiation in concert with BaP or promotion tumor formation in concert with TPA. However, there was no nicotine effect on either activity, even if TPA concentrations were varied. Bock concluded that the results of his experiments show that nicotine can enhance carcinogenesis induced by the combination of BaP and TPA, although the mechanism of this co-carcinogenesis and its relevance to humans remain unclear.

Supplementary Table 4 (continued)

Section #	Route of administration	Narrative
2	s.c.	Rana and Bhagat developed a so-called immunosympathectomized mouse model for their carcinogenesis studies (Rana & Bhagat 1970; Bhagat & Rana 1971). Newborn CF-1 mice were injected with an antiserum against a nerve growth factor, which is normally present in mouse sarcoma tissue and was suspected to play a critical role in carcinogenesis. In one study, on day 27 after birth, mice received s.c. injections of BaP (Bhagat & Rana 1971). Immunosympathectomized mice developed tumors slower than sham-treated mice. Similarly, mice sham-treated with a control serum and given s.c. injections of nicotine (0.5 mg/kg) thrice daily starting 3 d after BaP administration also showed delayed appearance of tumors. Both immunosympathectomy and nicotine treatment also caused lower tumor incidences. However, nicotine treatment resulted in a significant increase in the average weight of the tumors observed. In an earlier, most likely of very similar design but for which only an abstract is available, mice were s.c. treated with 1 mg/kg nicotine thrice daily (Rana & Bhagat 1970). In this study, nicotine did not show any effect on BaP-initiated carcinogenesis in either immunosympathectomized or control mice. The rationale for including nicotine in this study was not explained. Gurkalo and Volfson (1982) investigated the role of nicotine in a rat model of stomach carcinogenesis induced by N-methyl-*N'*-nitro-N-nitrosoguanidine (MNNG) given via the drinking water for 6 months. Nicotine was administered by twice weekly s.c. injections of 0.5 mg/kg. Because nicotine-related mortality was observed, the initial frequency of thrice weekly injections was reduced, and there were additional interruptions of several weeks throughout the 8-month nicotine treatment period reportedly to allow the rats to recover from nicotine toxicity. A control group with nicotine alone was omitted, as the researchers believed that nicotine under the circumstances of their experiment would not be carcinogenic (with reference to Truhaut & De Clercq 1961). The combined treatment with MNNG and nicotine resulted in an approximate doubling of the incidence of stomach tumors to 67% (10/15) from 30% (6/20) in the group with MNNG alone (Gurkalo & Volfson 1982). At the same time, some morphological changes in stomach glands seemed to be reduced in the MNNG/nicotine group compared to MNNG alone. The authors also suggested that the stomach tumors in the nicotine-treated group would develop earlier, although it does not seem that there were interim dissections. A few tumors at other sites were reported. The authors discussed the possible involvement of adrenergic mechanisms triggered by nicotine in the observed increase in carcinogenicity in this study. Historic controls from this laboratory are missing in order to judge the reproducibility of the stomach tumor incidence upon MNNG exposure. Control data from other laboratories cannot be used either because the rat strain used in this study is not known. Habs and Schmähl (1984) investigated the influence of nicotine on chemically induced mammary tumors in female Sprague-Dawley rats. Three months after a single i.v. dose of N-methyl-nitrosurea (MNU), a 100% incidence of mammary tumors was found. Nicotine given s.c. twice per week at an average dose of 0.04 mg/(kg × d) either during the week before MNU or for 3 months after MNU administration did not change tumor incidence, size, or histology. There was no effect by nicotine alone. The authors concluded that there was “no evidence that nicotine can influence chemical-induced tumors.” The study suffers from just using one high dose of the chemical carcinogen in combination with just one dose of nicotine. Schuller and colleagues exposed male Syrian golden hamsters s.c. to nicotine for their lifetime (approximately 16 months) at an average dose of 0.15 mg/(kg × d) in combination with 60% hyperoxia (Schuller et al. 1995). Hyperoxia induced severe morphological effects in the lungs, such as hemorrhages, interstitial edema, and emphysema, which would impair respiration. As such, this model was offered to reflect conditions in smokers with chronic lung disease. No tumors were observed when the hamsters were exposed to hyperoxia or nicotine alone. Two nasal adenocarcinomas, two lung adenomas, two lung adenocarcinomas, and one adrenal cortical adenocarcinoma were observed in the group of 20 hamsters exposed to both hyperoxia and nicotine. No statistics were reported. The tumors had areas staining positively for neuron-specific enolase as a marker for endocrine cells. The histological type of tumors seen in this study was reported to be different to those observed after a combined treatment with hyperoxia and *N*-nitrosamines. The authors concluded that the suggested “chronic stimulation of the nAChR in an environment of impaired pulmonary oxygenation contributes to the carcinogenic burden associated with exposure to cigarette smoke and provides selective growth advantage for lung tumors with neuroendocrine phenotype.” Bersch and colleagues implanted DMBA into the pancreatic head of CF1 mice and exposed them for 15 d before and 30 d after DMBA treatment to nicotine by s.c. injection (Bersch et al. 2009). The incidence of pancreatic adenocarcinomas was 52% in the nicotine-treated group. In a parallel group exposed to smoke inhalation at a concentration of 100 mg total particulate matter/m^3, the incidence was only 13%. Because there was neither a concurrent nor a historic sham s.c.-treated control for the nicotine exposure, the authors claimed a 17% incidence of pancreatic adenocarcinomas from a historic control treated with only DMBA. The authors suggested that nicotine is a powerful agent to promote intraepithelial lesions in the model used in this study. Hayashi and colleagues studied the role of nicotine in murine dextran sulfate sodium (DSS) induced colitis as well as in the colitis-associated

Supplementary Table 4 (continued)

Section #	Route of administration	Narrative
		cancer model induced by azoxymethane (Hayashi et al. 2014). Nicotine given s.c. at doses of 3 mg/(kg × d) to Balb/c mice during DSS treatment inhibited both colitis as well as colitis-associated cancer multiplicity. No effect on adenocarcinoma incidence was seen. Tumor size and the incidence of preneoplastic lesions in the proximal colon were reduced in the nicotine group. The authors considered that nicotine inhibited tumorigenesis via an anti-inflammatory effect in the colonic mucosa, which was mediated by nAChR and CD4+ lymphocytes. Berger and Zeller investigated a potential interference with the chemotherapy of two types of rat cancer models (Berger & Zeller 1988). In an MNU-induced model of mammary cancer, the chemotherapeutic effect of 1-(2-chloroethyl)-1-nitroso-3-(2-hydroxyethyl)urea was improved by nicotine administered with an osmotic minipump for 4 weeks at a dose of 5 mg/(kg × d) (no specification of route of administration, though presumably s.c.). No effect of nicotine on the MNU-induction of mammary carcinogenesis was observed.
2	i.p.	Habs and Schmähl (1976) used a model of transplacental carcinogenicity induced by N-ethyl-nitrosurea (ENU) administration to Sprague-Dawley rat dams and investigated the potential effect of post-natal nicotine administration. ENU induced a broad variety of benign and malignant tumors, which were mostly neurogenic but also included mammary or kidney tumors. Chronic nicotine administered i.p. at 2 mg/(kg × week) corresponding to an average of 0.3 mg/(kg × d) had no influence on the tumor incidences, latency periods, localization, or histology. Davis and colleagues used NNK-treated A/J mice as a model for pulmonary carcinogenesis (Davis et al. 2009). Nicotine administration (under conditions described for Treviño et al. 2012) for 7 months increased the multiplicity of lung tumors from approximately 10 to 15. The overall tumor area in these lungs more than doubled due to nicotine administration. Changes in gene expression were found, such as down-regulations for E-cadherin or β-catenin. The authors concluded that their results suggest that nicotine "can facilitate the progression and metastasis of tumors pre-initiated by tobacco carcinogens." It seems to be notable that in this publication several small errors in citations and experimental detail, such as group size, can be found, and a strict conceptual separation of *in vitro* and *in vivo* results and discussion is lacking. Iskandar and colleagues induced lung tumors in A/J mice by pretreatment with a single dose of NNK (Iskandar et al. 2013). Two weeks later, nicotine was i.p. administered thrice weekly for two months leading to an average dose of 0.4 mg/(kg × d). Nicotine co-exposure increased lung tumor multiplicity and volume by approximately two fold beyond NNK-induced tumorigenesis. No nicotine-only control was included. Interestingly, a parallel development of pulmonary emphysema was observed, and the morphological changes were statistically significantly increased by approximately 30% in the combined NNK/nicotine exposure group compared to the control group. The authors suggested that a "relevant animal lung cancer model for studying tumor growth within emphysematous microenvironments" was established. In a second experiment, the authors found that co-treatment with β-cyrptoxanthin inhibited "nicotine-promoted lung tumorigenesis and emphysema in A/J mice." Several molecular alterations were investigated in the mouse lung tissues along with the two disease endpoints, such as *IL-6* or *p*53 expression and SIRT1 levels, however, no histological characterization of the tumors was provided. It would have been interesting to see whether there was any effect on tumor progression from adenoma to adenocarcinoma in this study. In this second experiment, the authors apparently wanted to reproduce the findings of the first experiment (according to their Figure 1); however, the data shown in the bar graphs for control and NNK/nicotine groups are exactly the same (both means and standard errors) for all disease and molecular endpoints between the two experiments putting into question the independence of experiments 1 and 2.
3	i.v.	Paleari and colleagues intrathoracically implanted the lung tumor cell line A549 modified for chemiluminescence into NOD/SCID mice (n=10 to 12) and investigated the effects of daily injections of nicotine into their tail vein on tumor growth (Paleari et al. 2008). Although no quantitative data were shown, the authors claimed that the tumor incidence detectable by imaging the luminescent tumor cells increased from 60% in the control to 100% in the nicotine-treated group. In addition, it was claimed that an antagonist to the α_7-nAChR inhibited this tumor formation. Based on the limited information available on this study, a low-adequacy score was assigned, and it is difficult to judge the relevance of the results. Li and colleagues investigated the potential antagonist effect of nicotine on the chemotherapeutic effect of the epidermal growth factor receptor (EGFR) inhibitor erlotinib (Li et al. 2015). In one sub-study, nicotine was administered via i.v. injection at 5 days/week for 20 days after s.c. inoculation of PC9 NSCLC cells in nude BALB/c mice (group size unknown). An average nicotine dose of 0.04 mg/(kg × d) was estimated. Serum cotinine levels of 370 to 500 ng/ml were found when tested 30 min after the i.v. injection of nicotine. The authors did not explain why their control mice also had cotinine levels of up to 20 ng/ml, though. A small by statistically significant increase in tumor volume was observed. Interestingly, a parallel group of mice was exposed to nicotine via the drinking water resulting in an estimated daily dose of 20 mg/kg, and in this group a similar increase in tumor growth was observed as with the i.v. nicotine administration. Between days 21 and 36, erlotinib was additionally administered and inhibited the further growth of the xenograft tumors. Growth inhibition was less effective in the

Supplementary Table 4 (continued)

Section #	Route of administration	Narrative
		group with prior and concomitant oral nicotine exposure compared to that with i.v. injections. Nevertheless, in comparison to a control without nicotine, either nicotine treatment was attenuating the growth-inhibitory effect of erlotinib.
3	oral	Heeschen et al. (2001) used a s.c. Lewis carcinoma cell xenograft model based on C57Bl6 mice to study the potential effects of nicotine-inducible angiogenesis. Nicotine administration via the drinking water at an estimated dose of 20 mg/(kg × d) resulted in accelerated tumor growth. This was associated with increased vascularity in the tumor tissue. These effects could be observed after only one week of nicotine treatment. Practically the same results were obtained when the carcinoma cells were implanted into the lungs of the mice, although this sub-study had to be terminated after 12 d due to the rapid growth of the tumors. Based on parallel *in vitro* assays, the authors suggested that the observed effects were mediated through nAChR and occurred at a nicotine concentration that would be pathophysiologically relevant. No nicotine or nicotine metabolite levels in tissue or body fluids were reported in the *in vivo* study. In a separate study, the growth of s.c. carcinoma cells in this model could be inhibited by concomitant administration of mecamylamine, an nAChR antagonist (Heeschen et al. 2002, no nicotine administration in this study). Natori and colleagues administered nicotine daily by gavage to mice at a dose of 20 mg/kg for several days before and after s.c. inoculation with colon cancer cells (Natori et al. 2003). Accelerated tumor growth as well as increased tumor vascularization were observed. Few experimental details were reported. Al-Wadei and colleagues administered a pancreatic duct adenocarcinoma cell line s.c. to nude athymic mice followed by exposure to nicotine via the drinking water for 1 month (Al-Wadei et al. 2009). As in several other studies, the chemical composition of the nicotine added to the drinking water application was not disclosed. However, based on a later publication from this group (Banerjee et al. 2013), it can be deduced that nicotine bitartrate (NBT) was used. Based on the reported drinking water consumption and an estimated body weight, a dose of approximately 19 mg/(kg × d) was estimated. No signs of toxicity or body weight changes were reported. Tumors were growing to an approximately 4-fold greater volume in nicotine-exposed mice than in the respective sham-treated mice. The authors concluded that “these data suggest a strong tumor-promoting effect of nicotine.” If these mice were i.p. injected with γ-aminobutyric acid (GABA) during the course of the study, the growth of tumors from the inoculated cells was inhibited, independent of the presence of nicotine. The laboratory conducting this study has long suggested an effect of nicotine on tumorigenesis via the systemic release of catecholamines (Schuller 2007; Schuller 2014). The downstream mediator of catecholamines is cyclic adenosine 3',5'-monophosphate (cAMP), which can be antagonized by GABA. Thus, the current study suggests that nicotine can accelerate tumorigenesis via adrenergic (stress response) mechanisms (Al-Wadei et al. 2009). Lee and colleagues used two rather complicated test designs to investigate the potential effect of nicotine and the α9-nAChR in xenografts of transformed breast epithelial cells (Lee et al. 2010). In the first part, human mammary gland adenocarcinoma cells were stably transfected with α9-nAChR short interfering RNA (siRNA) to attenuate effects that might be mediated by this receptor. Controls were untreated cells and cells treated with scrambled siRNA. Groups of Balb/c NOD-SCID mice were inoculated (s.c.) with the three cell types, and tumor growth was observed for six weeks. While the α9-nAChR siRNA transfected cells grew smaller tumors than the two control cells, exposure of the three sub-groups to nicotine via the drinking water did not produce statistically significant differences in tumor weight. However, there was a slight increase in tumor volume and weight at the end of the 6-week nicotine administration. In the second part of the study, a normal human breast epithelial cell line was used which was transgenic for conditional overexpression of α9-nAChR. Upon chronic in vitro exposure to nicotine, a few of the α9-nAChR overexpressing cells were transformed to anchorage-independent growth in a soft agar assay. The latter cells were inoculated (s.c.) into NOD-SCID Balb/c mice to test for tumorigenicity with and without receptor overexpression and with and without nicotine administered in the drinking water. After five weeks, the receptor-overexpressing cells were grown to larger tumors than their respective controls. In addition, nicotine administration resulted in a statistically significant acceleration of tumor growth in both types of cell lines regardless of receptor overexpression. A concern with this study is that the reported nicotine dosing concentration (10 mg/ml in drinking water) seems to be totally out of range with other mouse studies (0.2 to 0.5 mg/ml). In studies using more than 1 mg/ml nicotine dosing concentration in drinking water, the animals showed signs of toxicity and drastically decreased their water consumption (Pietilä et al. 1995; Grabus et al. 2005), however body weight or water consumption data were not reported in this publication. Because no signs of toxicity were reported by Lee et al. (2010) at a 20- to 50-fold higher nicotine dosing concentration, it is difficult to interpret the findings of this study. Al-Wadei and colleagues more recently studied the effect of nicotine on xenografts of two types of human non-small cell lung carcinoma cells in nude mice (Al-Wadei et al. 2012). Nicotine treatment for 1 month was shown to stimulate the growth of the xenografts, which was accompanied by increased levels of α4 and α7 subunits of the nAChR and by activation of growth signaling pathways in the tumor tissue.

Supplementary Table 4 (continued)

Section #	Route of administration	Narrative
		Nicotine exposure further resulted in enhanced levels of catecholamine and cortisol as well as cAMP in serum and xenograft tissues. Again, GABA reversed the growth stimulus of nicotine and its effects on signaling pathways. The two cell lines produced similar results. They were both derived from human adenocarcinomas but different in terms of their K*ras* mutation status. For the actual nicotine concentration in the drinking water, vastly contradictory information was provided in the publication (1 µmol/l in the methods section and 200 mg/ml in the legend to the figure displaying tumor volumes). No clarification was obtained from the corresponding author; therefore, because all other study design parameters including comments on the relevance of the nicotine doses achieved were parallel to a previous study by these authors (Al-Wadei et al. 2009), the same water concentration of NBT used in the previous study (200 µg/ml) was assumed for the current study (Al-Wadei et al. 2012) yielding an estimated nicotine dose of 14 mg/(kg × d). Nakada and colleagues used Lewis carcinoma cell inoculation in C57Bl6 mice and investigated the effect of nicotine administered via the drinking water at an estimated dose of 20 mg/(kg × d) on tumor growth (Nakada et al. 2012). A weak stimulation of tumor growth was observed. It should be noted that the drinking water solutions were fortified with saccharin. No information on group size was given. In the latest studies from this group of researchers, Banerjee and colleagues used pancreatic duct adenocarcinoma cells in combination with a very low nicotine exposure in order to avoid an outright promotion of tumor growth and to study effects on therapeutic interactions (Banerjee et al. 2013; Banerjee et al. 2014). Athymic nude mice s.c. inoculated with the cancer cells were exposed to nicotine via the drinking water to obtain an estimated daily dose of 0.1 to 0.2 mg/kg. There was no effect on xenograft growth, as intended, but nicotine significantly reduced the therapeutic response of the xenografts to gemcitabine. This reduction in therapeutic response could be counteracted by GABA. Khalil and colleagues administered glioma cells intracranially into nude mice, which were exposed to nicotine via the drinking water at a reported concentration of 5 mg/ml, corresponding to approximately 1000 mg/(kg x d) (Khalil et al. 2013). This dose is extremely high, but no signs of toxicity were reported. The growth of brain tumors was apparently increased in mice treated with nicotine, although no statistical evaluation was provided. This study was scored as low adequacy. Liu and colleagues performed a xenograft study with nicotine as part of a mechanistic study on the effects of a Chinese plant extract as a potential chemopreventive agent (Liu et al. 2015). A549 cells were s.c. implanted, and nicotine was administered via the drinking water at an estimated dose of 0.03 mg/(kg × d) for 20 days. Nicotine accelerated the tumor growth, which was inhibitable by the concomitant administration of the plant extract. In the tumor tissue, nicotine seemed to be anti-apoptotic. Interestingly, the same apparent concentration of nicotine in the drinking water was considered to be below an effective level for inducing xenograft growth in a pancreatic cancer model (Banerjee et al. 2014).
3	dermal	Davis and colleagues more recently used an NRT patch on the shaved back of BALB/c mice after having mouse adenocarcinoma cells inoculated (s.c.) at the same position (Davis et al. 2009). A daily nicotine dose of 25 mg/(kg × d) was estimated. Over the course of 2 weeks, tumors in nicotine-exposed mice grew faster than those in unexposed mice. Apparently, there was no sham treatment with a vehicle in place of the nicotine solution, nor were the group sizes reported in this study.
3	s.c.	Warren and colleagues investigated the role of s.c. nicotine administration on the effectiveness of radio- and chemotherapy in an athymic nude mouse model with s.c. inoculated human lung cancer cells (Warren et al. 2012). Nicotine at an average dose of 0.9 mg/(kg × d) had no influence on the growth of these tumor cells. When the tumors were grown up to a size of 5 mm in one dimension, they underwent radiotherapy or radiotherapy in combination with chemotherapy. When nicotine was present during these therapies and in the days thereafter, the xenografts grew faster than without nicotine. It did not matter whether nicotine was actually given over a period of 6 or 28 d. The authors concluded that this was the first *in vivo* model confirming earlier *in vitro* indications of an impaired efficacy of cancer therapies by nicotine co-exposure.
3	i.p.	Davis and colleagues used a mouse adenocarcinoma cell line, which was s.c. implanted into BALB/c mice (Davis et al. 2009). Nicotine was administered by i.p. injections for 18 d at an average dose of 0.4 mg/(kg × d). This resulted in an increased tumor growth. When the tumors were surgically removed and the nicotine administration continued for another two weeks, the percentage of tumors recurring relative to those removed was 4-fold higher in nicotine- vs. sham-exposed mice. In this sub-study, there were also more lung metastases as well as larger areas covered with metastatic foci in the nicotine group. Maier and colleagues, in response to the prior study, inoculated AB6F1mice with cell lines derived from NNK-induced lung adenocarcinoma of this strain (Maier et al. 2011). Nicotine was administered by i.p. injection for 18 d at approximately 0.8 mg/kg. It is not stated whether injections were daily or less often per week. Acute toxicity was observed. No nicotine effect on tumor growth was found.

Supplementary Table 4 (continued)

Section #	Route of administration	Narrative
		Molfino and colleagues exposed Fischer rats to nicotine doses of approximately 100 mg/kg for three consecutive days within a 3-week study in order to investigate the potential protective effect of nicotine on tumor-associated anorexia-cachexia (Molfino et al. 2011). Tumors were induced by i.p. administration of MCA sarcoma cells. Nicotine at this high dose had no influence on tumor growth. The authors suggest that the tumor-induced body weight wasting syndrome might have been ameliorated by nicotine. Serum IL-1 levels were reduced, and there was no change in IL-6. Due to the small group size and the use of only a single i.p. nicotine dose, this study is of low adequacy. Treviño and colleagues inoculated athymic nude SCID mice with pancreatic duct cancer cells into the pancreas sub-capsular region (Treviño et al. 2012). The mice were exposed to nicotine by i.p. injection thrice weekly for one month resulting in an average daily dose of 0.4 mg/kg. The pancreatic tumor volume determined by marking tumor cells with luciferase was doubled in the nicotine group, and more liver metastases were observed compared to control. Nicotine exposure also inhibited the chemotherapeutic effect of gemcitabine on the pancreatic tumors in this model. The nicotine effects on tumor growth were reproduced in a second experiment within this study, which also showed that abrogation of the transcription factor inhibitor of differentiation-1 (Id1) by RNA interference inhibited both spontaneous as well as nicotine-affected tumor growth and metastasis. Pillai and colleagues were interested in the role of β-arrestin-1 mediating nicotine-induced effects (Pillai et al. 2015). As part of a series of mechanistic tests, A549 cells were orthotopically implanted into the left lung of SCID-beige mice. For the detection of tumor growth and potential metastases, these A549 cells were transfected with the luciferase gene. In order to study the role of β-arrestin-1, the respective gene was knocked down using shRNA before injection into a sub-group of mice. Nicotine injections (i.p., no dose given) for 7 weeks accelerated the growth of the A549-derived lung tumors compared to controls without nicotine (unclear whether there was a sham injection) in mice treated with a control shRNA. In addition, a trend towards more metastases in the brain, adrenals, and liver was seen (no statistical information provided). In those mice implanted with A549 cells with knocked down β-arrestin-1, nicotine administration did neither accelerate lung tumor growth nor lead to metastases. In the lung tumor tissue, higher levels of fibronectin and vimentin were found upon nicotine treatment, which is indicative of an epithelial-to-mesenchymal transition induced by nicotine. Yuge and colleagues investigated a potential interference of nicotine with chemotherapeutic treatments against the growth of T24 human bladder cancer cells that were s.c. implanted in a Matrigel matrix into the flanks of nude athymic BALB/c mice (n=10) (Yuge et al. 2015). When tumor masses were grown to 150 mm^3, drug treatment started. Nicotine administered i.p. for three times per week at a dose of 1 mg/kg (averaging to a nominal dose of 0.4 mg/(kg x d)) enhanced tumor growth by approximately twofold within the 21 day exposure period. A daily administration of a PI3K/mTOR dual inhibitor attenuated tumor growth both in the absence and presence of nicotine. Cis-platin given once at the beginning of the 21-d period inhibited tumor growth in the absence of nicotine, but exposure to nicotine prevented this chemotherapeutic effect. These studies were supplemented with immunohistochemical as well as in vitro studies demonstrating the relevance of the PI3K-dependent growth signaling pathway in this model. Improgo and colleagues used osmotic mini-pumps to administer nicotine of unknown chemical composition, presumably via the i.p. route, at a nominal daily dose of 24 mg/kg and found an approximately 4-fold increase in tumor weight developed from the small-cell cancer cell line DMS-53 s.c. injected into the hind flanks of athymic nude mice in comparison to a saline control (Improgo et al. 2013).
4	oral	Freedlander and French (1956) investigated the potential interaction of nicotine-*N*'-oxide (NNO) on the urethane-induced lung tumorigenicity in strain A mice. NNO was administered via the drinking water (unknown doses). After 4 months, the incidence of pulmonary adenomas in the NNO-exposed mice was reportedly similar to that observed in those only treated with urethane. Schmähl and Osswald (1968) examined the reproducibility of previous findings and administered cotinine in the drinking water to Wistar rats at an estimated dose of 30 mg/(kg × d) for 21 months. No indication of carcinogenesis was found in comparison to controls (probably historic controls); only 1/60 malignant liver tumor was seen in the cotinine-treated group. The authors concluded that cotinine is not carcinogenic to rats, and they believed that their study had an overall higher dose of cotinine than in the previous study. Nakada and colleagues inoculated (s.c.) C57Bl6 mice with Lewis carcinoma cells and subsequently exposed to cotinine at an estimated daily dose of 20 mg/(kg × d) via the drinking water (with saccharine) (Nakada et al. 2012). After 14 d, the tumors were significantly larger (2.3-fold) in cotinine- vs. sham-exposed mice. This effect was similar in size to that observed in a parallel group exposed to nicotine via the drinking water at the same dose.

Supplementary Table 4 (continued)

Section #	Route of administration	Narrative
4	s.c.	For the study of Boyland (1968), no experimental details on the strain of mice, the group sizes, the cotinine doses, and the duration of the various sub-studies are available. Also, the data table in this report is difficult to interpret. There is no indication of concurrent control groups. Cotinine was administered s.c. by implanting a pellet into the bladder by sub-scapular injection to neonatal mice, and by skin painting. Tumor numbers per number of surviving mice were reported. There were no tumors after s.c. administration and skin painting. The author stated that “the only possible tumorigenic action is the induction of 11 adenomas of the bladder in 69 mice in which pellets of cholesterol containing 10% cotinine were implanted into the urinary bladders.” The carcinomas observed in the same sub-study were not further discussed. Tumors were also observed in neonatally exposed mice but apparently not considered relevant by the author. Thus, in the absence of appropriate control groups, especially for the bladder studies, the relevance of these studies cannot be evaluated. No later full publication of these data was found.

i.p., intraperitoneal; i.v., intravenous; s.c., subcutaneous.