

Toxicological profile for

Nicotine

***This ingredient has been assessed to determine potential human health effects for the consumer. It was considered not to increase the inherent toxicity of the product and thus is acceptable under conditions of intended use.***

# *1.* *Name of substance and physico-chemical properties*

## *1.1.* *IUPAC systematic name*

3-[(2S)-1-methylpyrrolidin-2-yl]pyridine (PubChem)

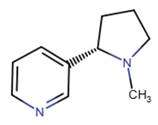
## *1.2.* *Synonyms*

(-)-3-(1-Methyl-2-pyrrolidyl)pyridine; (-)-Nicotine; (S)-3-(1-Methyl-2-pyrrolidinyl)pyridine; (S)-Nicotine; 1-Methyl-2-(3-pyridyl)pyrrolidine; 3-(1-Methyl-2-pyrrolidinyl)pyridine; 3-(N-Methylpyrrolidino)pyridine; beta-Pyridyl-alpha-N-methyl pyrrolidine; beta-Pyridyl-alpha-N-methylpyrrolidine; AI3-03424; Black leaf; Black leaf 40; Caswell No. 597; CCRIS 1637; Destruxol orchid spray; EINECS 200-193-3; Emo-nik; ENT 3,424; EPA Pesticide Chemical Code 056702; Exodus; Flux MAAG; Fumetobac; Habitrol; HSDB 1107; L-3-(1-Methyl-2-pyrrolidyl)pyridine; L-Nicotine; Mach-Nic; Micotine; Niagara P.A. dust; Nic-Sal; Nicabate; Nico-dust; Nico-fume; Nicocide; Nicoderm; Nicoderm CQ; Nicorette; Nicotin; Nicotina [Italian]; Nicotine alkaloid; Nicotine polacrilex; Nicotrol; Nicotrol Inhaler; Nicotrol NS; Nikotin [German]; Nikotyna [Polish]; Niquitin; NSC 5065; Ortho N-4 and N-5 dusts; Ortho N-4 dust; Ortho N-5 dust; Prostep; Pyridine, 3-(tetrahydro-1-methylpyrrol-2-yl); Pyrrolidine, 1-methyl-2-(3-pyridal)-; RCRA waste number P075; Tabazur; Tendust; Tetrahydronicotyrine, dl-; UNII-6M3C89ZY6R; XL all insecticide; Pyridine, 3-((2S)-1-methyl-2-pyrrolidinyl)-; Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)- (9CI); Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-, UN1654 (ChemIDplus).

## *1.3.* *Molecular formula*

C10H14N2 (ChemIDplus)

## *1.4.* *Structural Formula*

**

## *1.5.* *Molecular weight (g/mol)*

162.23156 g/mol

## *1.6.* *CAS registration number*

54-11-5

## *1.7.* *Properties*

### *1.7.1.* *Melting point*

(°C): -79 (ChemIDplus; EPISuite, 2017); -80 to -79 (ChemSpider)

### *1.7.2.* *Boiling point*

(°C): 247 (ChemIDplus; EPISuite, 2017); 244.4-248 or 264-270 (ChemSpider)

### *1.7.3.* *Solubility*

1.00E+06 mg/L in water (ChemIDplus; EPISuite, 2017); Soluble to 100 mM in water (ChemSpider)

### *1.7.4.* *pKa*

3.1 (ChemIDplus); 8.5 (PubChem)

### *1.7.5.* *Flashpoint*

(°C): 101 or 230 (ChemSpider), 95 or 101 (PubChem)

### *1.7.6.* *Flammability limits (vol/vol%)*

LOWER 0.7% BY VOL; UPPER 4.0% BY VOL (PubChem)

### *1.7.7.* *(Auto)ignition temperature*

(°C): 471°F (244) (PubChem)

### *1.7.8.* *Decomposition temperature*

(°C): 482°F (250) (PubChem)

### *1.7.9.* *Stability*

Nicotine is photosensitive and will gradually turn brown when exposed to light or air (PubChem)

### *1.7.10.* *Vapor pressure*

0.038 mmHg at 25°C (EPISuite, 2017, PubChem), 0.08 mmHg (PubChem)

### *1.7.11.* *log Kow*

1.17 (ChemIDplus; EPISuite, 2017; PubChem)

# *2.* *General information*

## *2.1.* *Exposure*

“This study investigates the transfer of nicotine from lactating dams to their offspring through breast milk, in the frame of a research focused to ascertain toxicological and neuro-behavioural effects on pups as consequence of either unavoidable ("yoked & forced") or voluntary ("freely-chosen") maternal nicotine exposure. To this aim, plasmatic concentrations of nicotine and cotinine were determined by LC-MS/MS in Wistar rat pups whose mothers were orally administered with nicotine during lactation. Mothers were divided into a voluntary drinking group, an unavoidable consumption group, and controls. The limits of detection and quantification of the LC-MS/MS method were 0.20 and 0.65 ng/mL, respectively. Within-laboratory reproducibility (CV%) was <12%, with recovery of 86.2-118.8%. Results showed the presence of nicotine in 67% of samples from freely-chosen consumption group (1.30 ± 0.31 ng/mL) and in 60% of samples from yoked-consumption group (1.19 ± 0.62 ng/mL); cotinine was found in all the samples from freely-chosen (1.92 ± 0.77 ng/mL) and yoked-consumption groups (1.43 ± 0.30 ng/mL). Data provide an evidence-based support to maternal/offspring nicotine transfer as function of different ways of oral exposure.” (Famele,et al. 2018)

“Nicotine in mainstream aerosol from heated tobacco products reached 70%–84% of the nicotine detected in smoke from reference cigarettes.

The tested heated tobacco products delivered more nicotine in aerosol than a cigalike e-cigarette and less nicotine than a tank style e-cigarette.

Nicotine delivery has been shown to vary considerably across the variety of EC products.” (Public Health England, 2018)

Nicotine is listed as an ingredient in a landscape/yard (0.35%) and a pesticide (0.5%) product by the US Department of Health and Human Services (2017).

“Recently, a variety of new tobacco-free-nicotine, TFN, products have been commercialized as e-liquids. Tobacco-derived nicotine contains predominantly (S)-(-)-nicotine, whereas TFN products may not. The TFN products are said to be cleaner, purer substances, devoid of toxic components that come from the tobacco extraction process. A variety of commercial tobacco and TFN products were analyzed to identify the presence and composition of each nicotine enantiomer. A rapid and effective enantiomeric separation of nicotine has been developed using a modified macrocyclic glycopeptide bonded to superficially porous particles. The enantiomeric assay can be completed in <2 min with high resolution and accuracy using high performance liquid chromatography with electrospray ionization mass spectrometry. The results of this study suggest the need for pharmacological studies of (R)-(+)-nicotine, which is present in much greater quantities in commercial TFN products compared to commercial tobacco-derived products. Such studies are required by the FDA for new enantiomeric pharmacological products.” (Hellinghausen et al. 2017).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Chemical Abstracts Registry Number | Skin | Name | Permissable Exposure Limit (PEL) | |
| ppm | mg/m3 |
| 54115 | S | Nicotine; 1-methyl-2-(3-pyridyl)-pyrrolidine | 0.075 | 0.5 |

The substances designated by “S” in the skin notation column may be absorbed into the bloodstream through the skin, the mucous membranes and/or the eye, and contribute to the overall exposure.

As taken from Cal/OSHA

|  |  |  |
| --- | --- | --- |
| **Substance** | Nicotine | |
| **CAS No.** | 54-11-5 | |
|  | Limit value - Eight hours | Limit value - Short term |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | ppm | mg/m³ | ppm | mg/m³ |
| Australia |  | 0,5 |  |  |
| Austria | 0,07 | 0,5 | 0,28 | 2 |
| Belgium |  | 0,5 |  |  |
| Canada - Ontario |  | 0,5 |  |  |
| Canada - Québec |  | 0,5 |  |  |
| Denmark |  | 0,5 |  | 1 |
| European Union |  | **0,5** |  |  |
| Finland |  | 0,5 |  | 1,5 (1) |
| France |  | 0,5 |  |  |
| Germany (AGS) |  | 0,5 (1) |  | 1 (1)(2) |
| Hungary |  | 0,5 |  |  |
| Ireland |  | 0,5 |  |  |
| Italy |  | 0,5 |  |  |
| Latvia |  | 0,5 |  |  |
| New Zealand |  | 0,5 |  |  |
| Poland |  | 0,5 |  |  |
| Romania |  | 0,5 |  |  |
| Singapore |  | 0,5 |  |  |
| South Korea |  | 0,5 |  |  |
| Spain |  | 0,5 |  |  |
| Sweden |  | 0,1 |  |  |
| Switzerland | 0,07 | 0,5 | 0,14 | 1 |
| The Netherlands |  | 0,5 |  |  |
| Turkey |  | 0,5 |  |  |
| USA - NIOSH |  | 0,5 |  |  |
| USA - OSHA |  | 0,5 |  |  |
| United Kingdom |  | 0,5 |  | 1,5 |
|  | Remarks | | | |
| European Union | Bold-type: Indicative Occupational Exposure Limit Values and Limit Values for Occupational Exposure Binding Occupational Exposure Limit Value - BOELV ~ (for references see bibliography) | | | |
| Finland | (1) 15 minutes average value | | | |
| France | Italic type: Indicative statutory limit values | | | |
| Germany (AGS) | (1) Inhalable aerosol and vapour (2) 15 minutes reference period | | | |
| Italy | skin | | | |
| Spain | skin | | | |

As taken from GESTIS

OCCUPATIONAL EXPOSURE LIMITS

OEL-ARAB Republic of Egypt: TWA 0.5 mg/m3, Skin, JAN1993

OEL-ICELAND: TWA 0.5 mg/m3, skin, NOV2011

OEL-MEXICO: TWA 0.5 mg/m3;STEL 1.5 mg/m3 (skin), 2004

OEL-NORWAY: TWA 0.5 mg/m3, JAN1999

OEL-PERU: TWA 0,5 mg/m3, JUL2005

OEL-THE PHILIPPINES: TWA 0.5 mg/m3, Skin, JAN1993

OEL-POLAND: MAC(TWA) 0.5 mg/m3, MAC(STEL) 1.5 mg/m3, JAN1999

OEL-SWEDEN: TWA 0.5 mg/m3, JUN2005

OEL-THAILAND: TWA 0.5 mg/m3, JAN1993

OEL IN ARGENTINA, BULGARIA, COLOMBIA, JORDAN check ACGIH TLV;

OEL IN SINGAPORE, VIETNAM check ACGIH TLV

|  |  |
| --- | --- |
| MSHA STANDARD-air:TWA 0.5 mg/m3 (skin) | DTLVS\* The Threshold Limit Values (TLVs) and Biological Exposure Indices (BEIs) booklet issues by American Conference of Governmental Industrial Hygienists (ACGIH), Cincinnati, OH, 1996 Volume(issue)/page/year: 3,181,1971 |
| OSHA PEL (Gen Indu):8H TWA 0.5 mg/m3 (skin) | CFRGBR Code of Federal Regulations. (U.S. Government Printing Office, Supt. of Documents, Washington, DC 20402) Volume(issue)/page/year: 29,1910.1000,1994 |
| OSHA PEL (Construc):8H TWA 0.5 mg/m3 (skin) | CFRGBR Code of Federal Regulations. (U.S. Government Printing Office, Supt. of Documents, Washington, DC 20402) Volume(issue)/page/year: 29,1926.55,1994 |
| OSHA PEL (Shipyard):8H TWA 0.5 mg/m3 (skin) | CFRGBR Code of Federal Regulations. (U.S. Government Printing Office, Supt. of Documents, Washington, DC 20402) Volume(issue)/page/year: 29,1915.1000,1993 |
| OSHA PEL (Fed Cont):8H TWA 0.5 mg/m3 (skin) | CFRGBR Code of Federal Regulations. (U.S. Government Printing Office, Supt. of Documents, Washington, DC 20402) Volume(issue)/page/year: 41,50-204.50,1994 |

As taken from RTECS, 2018

|  |  |
| --- | --- |
| ACGIH TLV | 0.5 mg/m3 Skin |
| OSHA PEL | 0.5 mg/m3 Skin |
| NIOSH REL | 0.5 mg/m3 Skin |

DFG MAK Skin

As taken from ACGIH, 2018a.

ACGIH TLV basis: Gastrointestinal damage; CNS impairment; cardiovascular impairment.

As taken from ACGIH, 2018b

National Occupational Exposure Survey (1981 - 1983)

Estimated Numbers of Employees Potentially Exposed to Specific Agents by Occupation\*

|  |  |
| --- | --- |
| Agent Name | NICOTINE |
| CAS # | 54-11-5 |
| RTECS # | QS5250000 |
| Agent Code | 50570 |

|  |  |  |  |
| --- | --- | --- | --- |
| Code | Occupation Description (1980) | Total # Employees (Male & Female) | Total # Female Employees |
| 766 | FURNACE, KILN, AND OVEN OPERATORS, EXC. FOOD | 4,737 | 861 |
| TOTAL | | 4,737 | 861 |

\*(1) The estimates for each occupation apply across the surveyed industries in which the agent was observed. Not all industries were surveyed, and not all agents were observed in all surveyed industries. (2) When using the estimates, standard errors associated with estimates should be considered. (3) Potential exposures to a chemical agent are categorized as actual (i.e., the surveyor observed the use of the specific agent) or tradename (i.e., the surveyor observed the use of a tradename product known to contain the specific agent). The estimates presented in the table combine both categories. As taken from NIOSH, available at: [https://web.archive.org/web/20111028153518/http://www.cdc.gov/noes/noes2/50570occ.html](https://web.archive.org/web/20111028153518/http:/www.cdc.gov/noes/noes2/50570occ.html)

“Used in medicine and as an insecticide; [ACGIH] Used in pesticides, e.g., Black Leaf 40 that contains 40% nicotine sulfate” (Haz-Map, 2017).

“Consumers of combustible cigarettes are exposed to many different toxicologically relevant substances associated with negative health effects. Newly developed "heat not burn" (HNB) devices are able to contain lower levels of Harmful and Potentially Harmful Constituents (HPHCs) in their emissions compared to tobacco cigarettes. However, to develop toxicological risk assessment strategies, further independent and standardized investigations addressing HPHC reduction need to be done. Therefore, we generated emissions of a commercially available HNB product following the Health Canada Intense smoking regimen and analyzed total particulate matter (TPM), nicotine, water, aldehydes, and other volatile organic compounds (VOCs) that are major contributors to health risk. We show that nicotine yield is comparable to typical combustible cigarettes, and observe substantially reduced levels of aldehydes (approximately 80-95%) and VOCs (approximately 97-99%). Emissions of TPM and nicotine were found to be inconsistent during the smoking procedure. Our study confirms that levels of major carcinogens are markedly reduced in the emissions of the analyzed HNB product in relation to the conventional tobacco cigarettes and that monitoring these emissions using standardized machine smoking procedures generates reliable and reproducible data which provide a useful basis to assess exposure and human health risks.” (Mallock et al. 2018)

“INTRODUCTION: Electronic cigarettes (e-cigarettes) are purported to deliver nicotine aerosol without any toxic combustion products present in tobacco smoke. In this longitudinal within-subjects observational study, we evaluated the effects of e-cigarettes on nicotine delivery and exposure to selected carcinogens and toxicants. METHODS: We measured seven nicotine metabolites and 17 tobacco smoke exposure biomarkers in the urine samples of 20 smokers collected before and after switching to pen-style M201 e-cigarettes for 2 weeks. Biomarkers were metabolites of 13 major carcinogens and toxicants in cigarette smoke: one tobacco-specific nitrosamine (NNK), eight volatile organic compounds (1,3-butadiene, crotonaldehyde, acrolein, benzene, acrylamide, acrylonitrile, ethylene oxide, and propylene oxide), and four polycyclic aromatic hydrocarbons (naphthalene, fluorene, phenanthrene, and pyrene). Changes in urine biomarkers concentration were tested using repeated measures analysis of variance. RESULTS: In total, 45% of participants reported complete abstinence from cigarette smoking at 2 weeks, while 55% reported continued smoking. Levels of total nicotine and some polycyclic aromatic hydrocarbon metabolites did not change after switching from tobacco to e-cigarettes. All other biomarkers significantly decreased after 1 week of using e-cigarettes (p < .05). After 1 week, the greatest percentage reductions in biomarkers levels were observed for metabolites of 1,3-butadiene, benzene, and acrylonitrile. Total NNAL, a metabolite of NNK, declined by 57% and 64% after 1 and 2 weeks, respectively, while 3-hydroxyfluorene levels declined by 46% at week 1, and 34% at week 2. CONCLUSIONS: After switching from tobacco to e-cigarettes, nicotine exposure remains unchanged, while exposure to selected carcinogens and toxicants is substantially reduced. IMPLICATIONS: To our knowledge, this is the first study that demonstrates that substituting tobacco cigarettes with an e-cigarette may reduce user exposure to numerous toxicants and carcinogens otherwise present in tobacco cigarettes. Data on reduced exposure to harmful constituents that are present in tobacco cigarettes and e-cigarettes can aid in evaluating e-cigarettes as a potential harm reduction device.” (Goniewicz et al. 2017)

“The Health Hazard Evaluation Program received a request from the owner of a vape shop who was concerned about employees' potential exposure to vaping chemicals in the workplace. We collected air samples in the vape shop for flavoring chemicals (diacetyl, 2,3-pentanedione, 2,3-hexanedione, acetaldehyde, and acetoin), nicotine, formaldehyde, and propylene glycol. We took wipe samples for nicotine and metals on commonly touched surfaces. We found that employees vaped in the shop throughout the day, but very few customers vaped. None of the airborne concentrations of the specific flavoring chemicals we measured were above applicable occupational exposure limits although we detected low levels of two flavoring chemicals, diacetyl and 2,3-pentanedione, in the personal and area air samples. We detected the presence of metals, such as chromium, lead, copper, and nickel, on surfaces in the shop. We found detectable levels of nicotine on the outside surface of a nicotine transfer bottle. This may have occurred when liquid was poured from one bottle to another without use of a funnel. We did not find nicotine on other surfaces that we sampled. We found that not all employees wore chemical protective gloves when handling liquids containing nicotine. The bottle of stock nicotine solution was stored in the same refrigerator used to store employees' food. We recommend that the employer implement a policy prohibiting vaping in the workplace with e-liquids that contain diacetyl and 2,3-pentanedione. We also recommended not storing chemicals in the same area where food is stored or eaten, training employees on proper chemical handling procedures, and inspecting and maintaining the shop's exhaust ventilation systems.”(Zwack et al. 2017)

## *2.2.* *Combustion products*

Not required.

## *2.3.* *Ingredient(s) from which it originates*

Nicotine is an alkaloid naturally occurring in wide variety of plants, especially belonging to the family of Solanaceae, such as tomatoes, aubergines, peppers and potatoes (Doolittle et al., 1995). However, the principal source of nicotine is tobacco (Nicotiana tabacum) where it occurs at concentrations ranging from 0.17 to 4.93% (Djordjevic and Doran 2009).

# *3.* *Status in legislation and other official guidance*

“Nicotine-containing liquid should only be allowed to be placed on the market under this Directive, where the nicotine concentration does not exceed 20 mg/ml. This concentration allows for a delivery of nicotine that is comparable to the permitted dose of nicotine derived from a standard cigarette during the time needed to smoke such a cigarette. In order to limit the risks associated with nicotine, maximum sizes for refill containers, tanks and cartridges should be set” (Tobacco Product Directive 2014/40/EU).

“The revised European Union Tobacco Products Directive is now fully operational in England, transposed into UK law through the UK Tobacco and Related Products Regulations 2016, and covers e-cigarettes and nicotine-containing e-liquids that do not have a medicinal licence. These regulations include a notification process to the Medicines Healthcare products Regulatory Agency (MHRA), minimum standards for safety and quality of e-cigarette products, standards for information provision (including a nicotine health warning) and advertising restrictions and updated standards. The Advertising Standards Authority has carried out a consultation on health claims; the results are awaited. A system to report side-effects and safety concerns related to e-cigarettes has been implemented.”

“Since May 2017, nicotine concentration in liquids has been limited to a maximum of 20mg/mL. In March 2017, around 6% of e-cigarette users reported using higher nicotine concentrations; substantial proportions had difficulties reporting these figures so more may have been affected by the limit.” (Public Health England, 2018)

ADI (Acceptable Daily Intake) 0.0001 mg/kg bw/day

ARfD (Acute Reference Dose) 0.0001 mg/kg bw/day

As taken from BPDB, 2018

Nicotine (CAS RN 54-11-5) is included on the New Zealand Inventory of Chemicals under approval status HSR003032 (NZ EPA, 2006) and is classified according to the Environmental Protection Authority of New Zealand (NZ EPA CCID).

|  |  |
| --- | --- |
| Substance | Nicotine and its salts |
| CAS # | 54-11-5 |
| EC # | 200-193-3 |
| INN/ISO/AN |  |
| Regulation | (EC) No 1223/2009 |
| Regulated By | 76/768/EEC |
| Other Directives/Regulations |  |
| Annex/Ref # | II/246 |
| SCCS opinions |  |
| Chemical/IUPAC Name | Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)- |
| Identified INGREDIENTS or substances e.g. | **(\*)** Sodium nicotinate; 3-Pyridinecarboxylic acid, sodium salt (1:1) |
| Note |  |
| Current Version | v.1 |

As taken from CosIng, available at <http://ec.europa.eu/growth/tools-databases/cosing/>

There is a REACH dossier on nicotine (ECHA, 2018a).

Nicotine (CAS RN 54-11-5) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008, whereas nicotine (ISO) 3-[(2S)-1-methylpyrrolidin-2-yl]pyridine (CAS RN 54-11-5) is classified (ECHA, 2018b).

Nicotine (CAS RN 54-11-5) is listed by the US EPA Office of Pesticide Programs (2018) and is registered as an antimicrobial and “conventional chemical” pesticide.

Nicotine (CAS RN 54-11-5) is included in the US EPA Toxic Substances Control Act (TSCA) inventory.

The TSCA inventory is available at <https://iaspub.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do>

# *4.* *Metabolism/Pharmacokinetics*

## *4.1.* *Metabolism/metabolites*

“RATIONALE: The ability of nicotine to suppress body weight is cited as a factor impacting smoking initiation and the failure to quit. Self-administered nicotine in male rats suppresses weight independent of food intake, suggesting that nicotine increases energy expenditure. OBJECTIVE: The current experiment evaluated the impact of self-administered nicotine on metabolism in rats using indirect calorimetry and body composition analysis. METHODS: Adult male rats with ad libitum access to powdered standard rodent chow self-administered intravenous infusions of nicotine (60 μg/kg/infusion or saline control) in daily 1-h sessions in the last hour of the light cycle. Indirect calorimetry measured respiratory exchange ratio (RER), energy expenditure, motor activity, and food and water consumption for 22.5 h between select self-administration sessions. RESULTS: Self-administered nicotine suppressed weight gain and reduced the percent of body fat without altering the percent of lean mass, as measured by Echo MRI. Nicotine reduced RER, indicating increased fat utilization; this effect was observed prior to weight suppression. Moreover, nicotine intake did not affect motor activity or energy expenditure. Daily food intake was not altered by nicotine self-administration; however, a trend in suppression of meal size, a transient suppression of water intake, and an increase in meal frequency was observed. CONCLUSION: These data provide evidence that self-administered nicotine suppresses body weight via increased fat metabolism, independent of significant changes in feeding, activity, or energy expenditure.” (Rupprecht et al. 2018)

“Common variation in the CYP2B6 gene, encoding the cytochrome P450 2B6 enzyme, is associated with substrate-specific altered clearance of multiple drugs. CYP2B6 is a minor contributor to hepatic nicotine metabolism, but the enzyme has been proposed as relevant to nicotine-related behaviors because of reported CYP2B6 mRNA expression in human brain tissue. Therefore, we hypothesized that CYP2B6 variants would be associated with altered nicotine oxidation, and that nicotine metabolism by CYP2B6 would be detected in human brain microsomes. We generated recombinant enzymes in insect cells corresponding to nine common CYP2B6 haplotypes and demonstrate genetically determined differences in nicotine oxidation to nicotine iminium ion and nornicotine for both (S) and (R)-nicotine. Notably, the CYP2B6.6 and CYP2B6.9 variants demonstrated lower intrinsic clearance relative to the reference enzyme, CYP2B6.1. In the presence of human brain microsomes, along with nicotine-N-oxidation, we also detect nicotine oxidation to nicotine iminium ion. However, unlike N-oxidation, this activity is NADPH independent, does not follow Michaelis-Menten kinetics, and is not inhibited by NADP or carbon monoxide. Furthermore, metabolism of common CYP2B6 probe substrates, methadone and ketamine, is not detected in the presence of brain microsomes. We conclude that CYP2B6 metabolizes nicotine stereoselectively and common CYP2B6 variants differ in nicotine metabolism activity, but did not find evidence of CYP2B6 activity in human brain.” (Bloom et al. 2019).

“Cytochrome P450 2A13 (CYP2A13) is responsible for the metabolism of chemical compounds such as nicotine, coumarin, and tobacco-specific nitrosamine. Several of these compounds have been recognized as procarcinogens activated by CYP2A13. We recently showed that CYP2A13\*2 contributes to inter-individual variations observed in bladder cancer susceptibility because CYP2A13\*2 might cause a decrease in enzymatic activity. Other CYP2A13 allelic variants may also affect cancer susceptibility. In this study, we performed an in vitro analysis of the wild-type enzyme (CYP2A13.1) and 8 CYP2A13 allelic variants, using nicotine and coumarin as representative CYP2A13 substrates. These CYP2A13 variant proteins were heterologously expressed in 293FT cells, and the kinetic parameters of nicotine C-oxidation and coumarin 7-hydroxylation were estimated. The quantities of CYP2A13 holoenzymes in microsomal fractions extracted from 293FT cells were determined by measuring reduced carbon monoxide-difference spectra. The kinetic parameters for CYP2A13.3, CYP2A13.4, and CYP2A13.10 could not be determined because of low metabolite concentrations. Five other CYP2A13 variants (CYP2A13.2, CYP2A13.5, CYP2A13.6, CYP2A13.8, and CYP2A13.9) showed markedly reduced enzymatic activity toward both substrates. These findings provide insights into the mechanism underlying inter-individual differences observed in genotoxicity and cancer susceptibility.” (Kumondai et al. 2018).

“Tobacco and alcohol are often co-abused. Nicotine can enhance alcoholic fatty liver, and CYP2A6 (CYP2A5 in mice), a major metabolism enzyme for nicotine, can be induced by alcohol. CYP2A5 knockout (cyp2a5-/-) mice and their littermates (cyp2a5+/+) were used to test whether CYP2A5 has an effect on nicotine-enhanced alcoholic fatty liver. The results showed that alcoholic fatty liver was enhanced by nicotine in cyp2a5+/+ mice but not in the cyp2a5-/- mice. Combination of ethanol and nicotine increased serum triglyceride in cyp2a5+/+ mice but not in the cyp2a5-/- mice. Cotinine, a major metabolite of nicotine, also enhanced alcoholic fatty liver, which was also observed in cyp2a5+/+ mice but not in the cyp2a5-/- mice. Nitrotyrosine and malondialdehyde (MDA), markers of oxidative/nitrosative stress, were induced by alcohol and were further increased by nicotine and cotinine in cyp2a5+/+ mice but not in the cyp2a5-/- mice. Reactive oxygen species (ROS) production during microsomal metabolism of nicotine and cotinine was increased in microsomes from cyp2a5+/+ mice but not in microsomes from cyp2a5-/- mice. These results suggest that nicotine enhances alcoholic fatty liver in a CYP2A5-dependent manner, which is related to ROS produced during the process of CYP2A5-dependent nicotine metabolism.” (Chen X et al. 2018).

“Acute exposure to nicotinic agonists induces myotoxicity in zebrafish embryos. The main goal of this work was to evaluate the potential myotoxicity of nicotine acetylcholine receptor agonists on adult zebrafish muscle tissue by using nicotine as a model compound. Liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) datasets were processed with different chemometric tools based on the selection of Regions of Interest (ROI) and Multivariate Curve-Resolution (ROI-MCR procedure) Alternating Least Squares (ALS) for the analysis of different exposure experiments. Analysis of Variance Simultaneous Component Analysis (ASCA) of changes on metabolite peak profile areas showed significant nicotine concentration and exposure time-dependent changes, clearly differentiating between exposed and non-exposed samples and between short (2 h) and long exposure times (6 h or 24 h). Most of the changes observed in the concentrations of different metabolites are probably secondary to the observed hyperlocomotion, as they have been also observed in humans after strenuous muscular exercise. The absence of myotoxicity might be related with the reduced calcium permeability of adult muscle-type nicotinic acetylcholine receptors (nAChRs).” (Gomez-Canela et al. 2018).

Metabolism of nicotine involves the microsomal oxidation (phase I metabolism) and N- and O-glucuronidation (phase II metabolism). In human body 70-80% of nicotine is metabolized to cotinine by C-oxidation and mostly mediated through the hepatic cytochrome P450 CYP2A6 (McKennis et al., 1957; Hucker et al., 1960; Nakajima et al., 1996; Messina et al., 1997). The lungs and kidneys are also partially involved in the metabolism of nicotine. A number of genotypes of CYP2A6 have been determined and a recent intravenous study (Benowitz et al., 2006) classified subjects in three phenotypes according to CYP2A6 activity. Elimination half-lives range from 1.8 to 2.9 hours between three phenotypes. Considering its short biological half-life in humans, no accumulation is foreseen.

“Although the metabolism of nicotine varies considerably between individuals, nicotine has a short-half life, approximately two hours which, together with the repeat high boli of nicotine resulting from puffing on tobacco cigarettes, enables users to self-titrate. Self-titration is also seen in EC users (86). The speed at which nicotine is metabolised is affected by a number of factors, and plays a role over and above the rate at which it is absorbed and delivered to the brain and the dose received.” (Public Health England, 2018).

“OBJECTIVES: Smoking patterns and cessation rates vary widely across smokers and can be influenced by variation in rates of nicotine metabolism [i.e. cytochrome P450 2A6 (CYP2A6), enzyme activity]. There is high heritability of CYP2A6-mediated nicotine metabolism (60-80%) owing to known and unidentified genetic variation in the CYP2A6 gene. We aimed to identify and characterize additional genetic variants at the CYP2A6 gene locus. METHODS: A new CYP2A6-specific sequencing method was used to investigate genetic variation in CYP2A6. Novel variants were characterized in a White human liver bank that has been extensively phenotyped for CYP2A6. Linkage and haplotype structure for the novel single nucleotide polymorphisms (SNPs) were assessed. The association between novel five-SNP diplotypes and nicotine metabolism rate was investigated. RESULTS: Seven high-frequency (minor allele frequencies ≥6%) noncoding SNPs were identified as important contributors to CYP2A6 phenotypes in a White human liver bank (rs57837628, rs7260629, rs7259706, rs150298687 (also denoted rs4803381), rs56113850, rs28399453, and rs8192733), accounting for two times more variation in in-vitro CYP2A6 activity relative to the four established functional CYP2A6 variants that are frequently tested in Whites (CYP2A6\*2, \*4, \*9, and \*12). Two pairs of novel SNPs were in high linkage disequilibrium, allowing us to establish five-SNP diplotypes that were associated with CYP2A6 enzyme activity (rate of nicotine metabolism) in-vitro in the liver bank and in-vivo among smokers. CONCLUSION: The novel five-SNP diplotype may be useful to incorporate into CYP2A6 genotype models for personalized prediction of nicotine metabolism rate, cessation success, and response to pharmacotherapies.” (Tanner et al. 2018).

“AIMS: Nicotine addiction is an issue faced by millions of individuals worldwide. As a result, nicotine replacement therapies, such as transdermal nicotine patches, have become widely distributed and used. While the pharmacokinetics of transdermal nicotine have been extensively described using noncompartmental methods, there are few data available describing the between-subject variability in transdermal nicotine pharmacokinetics. The aim of this investigation was to use population pharmacokinetic techniques to describe this variability, particularly as it pertains to the absorption of nicotine from the transdermal patch. METHODS: A population pharmacokinetic parent-metabolite model was developed using plasma concentrations from 25 participants treated with transdermal nicotine. Covariates tested in this model included: body weight, body mass index, body surface area (calculated using the Mosteller equation) and sex. RESULTS: Nicotine pharmacokinetics were best described with a one-compartment model with absorption based on a Weibull distribution and first-order elimination and a single compartment for the major metabolite, cotinine. Body weight was a significant covariate on apparent volume of distribution of nicotine (exponential scaling factor 1.42). After the inclusion of body weight in the model, no other covariates were significant. CONCLUSIONS: This is the first population pharmacokinetic model to describe the absorption and disposition of transdermal nicotine and its metabolism to cotinine and the pharmacokinetic variability between individuals who were administered the patch.” (Linakis et al. 2017).

## *4.2.* *Absorption, distribution and excretion*

“Introduction: Novel nicotine delivery systems represent an evolving part of the tobacco harm reduction strategy. The pharmacokinetic (PK) profile of nicotine delivered by P3L, a pulmonary nicotine delivery system, and its effects on smoking urges and craving relief in relation to Nicorette inhalator were evaluated. Methods: This open-label, ascending nicotine levels study was conducted in 16 healthy smokers. Three different nicotine delivery levels, 50, 80, and 150 µg/puff, delivered by the P3L system were evaluated consecutively on different days after the use of the Nicorette inhalator. Venous nicotine PK, subjective effects, and tolerability were assessed. Results: Geometric least-squares means for maximum plasma nicotine concentration (Cmax), generated by the mixed-effect model for exposure comparison, were 9.7, 11.2, and 9.8 ng/mL for the 50, 80, and 150 µg/puff P3L variants, respectively, compared to 6.1 ng/mL after Nicorette inhalator use. Median time from product use start to Cmax was 7.0 minutes for all P3L, compared to 30.0 minutes for the Nicorette inhalator. Craving reduction was slightly faster than with the Nicorette inhalator as assessed with the visual analog scale craving score. The mean Questionnaire of Smoking Urges -brief total scores did not differ for both products. P3L was well tolerated. Conclusions: At all three nicotine levels tested, the inhalation of the nicotine lactate aerosol delivered with the P3L provided plasma nicotine concentrations higher and faster compared to the Nicorette inhalator. The plasma nicotine concentration-time profile supports a pulmonary route of absorption for P3L compared to the oromucosal absorption of the Nicorette inhalator. Implications: The combination of nicotine and lactic acid with the P3L device shows potential over existing nicotine delivery systems by delivering nicotine with kinetics close to published data on conventional cigarettes and without exogenous carrier substances as used in current electronic nicotine delivery systems. Altogether, the PK profile, subjective effects, and safety profile obtained in this study suggest P3L is an innovative nicotine delivery product that will be acceptable to adult smokers as an alternative to cigarettes.” (Teichert et al. 2018).

“E-cigarettes are battery-powered electronic devices from which users can inhale nicotine following its aerosolisation from a liquid solution. Some regulators and public health bodies consider e-cigarettes as potentially playing a major role in tobacco harm reduction. Their ability to provide nicotine to smokers in both amount and in a manner and form generally similar to cigarette smoking have been proposed as key components to help smokers reduce or cease the use of combustible cigarettes. Nicotine pharmacokinetic studies of e-cigarettes have been performed for a number of years and are beginning to show how nicotine delivery is evolving as the products themselves evolve. In this review, we provide a critical overview of the literature to describe what is known about nicotine delivery from e-cigarettes. We will discuss how the progression of e-cigarette design, development, and user familiarity has allowed increases in nicotine availability to the user, in the context of how much and how rapidly nicotine is delivered during acute-use periods. This review will also provide insight into current research gaps and highlight the potential utility of modelling and the standardisation of methodologies used to assess nicotine delivery to facilitate identification of products that are best suited to displace cigarette smoking among adult smokers.” (Fearon et al. 2018).

“Application of heat (hyperthermic conditions) on skin is known to enhance drug transfer and facilitate skin penetration of molecules. The aim of this work was to study the effect of hyperthermia on the drug release and skin permeation from nicotine transdermal patches. The drug release and skin permeation were characterized by in vitro release test and in vitro permeation test. The temperature was maintained at 32°C as control (simulating normal physiological skin temperature) and 42 C as hyperthermia condition. The in vitro release test was carried out using USP apparatus 5-Paddle over disk method for a transdermal patch. Skin permeation study was carried out across porcine skin using the flow through cells (PermeGear, Inc.) with an active diffusion area of 0.94 cm2. Mechanistic studies (parameters such as partition coefficient, TEWL and electrical resistivity) were also performed to understand the mechanisms involved in determining the influence of hyperthermia on drug delivery from transdermal patches of nicotine. The rate and extent of drug release from nicotine patch was not significantly different at two temperatures (Cumulative release after 12 h was 43.99 ± 3.29% at 32 C and 53.70 ± 5.14% at 42 C). Whereas, in case of in vitro permeation studies, the nicotine transdermal permeation flux for patch was threefold higher at 42 C (100.1 ± 14.83 μg/cm2/h) than at 32 C (33.3 ± 14.83 μg/cm2/h). The mechanistic studies revealed that the predominant mechanism of enhancement of drug permeation by hyperthermia condition is by the way of increasing the skin permeability. There is a potential concern of dumping of higher dose of nicotine via transdermal route.” (Panda et al. 2019).

“This publication is part of a series of 3 publications and describes the clinical assessment performed to fulfill the regulatory requirement per Art. 6 (2) of the EU Tobacco Products Directive 2014/40/EU under which Member States require manufacturers and importers of cigarettes and Roll Your Own tobacco containing an additive that is included in the priority list established by Commission Implementing Decision (EU) 2016/787 to carry out comprehensive studies (European Union, 2016). In our clinical study, two distinct end points were investigated, namely measuring plasma nicotine pharmacokinetics as a measure of nicotine uptake, and analyses of changes in smoker puffing behavior as a measure of cigarette smoke inhalation. This clinical study indicated that the inclusion of none of the priority additives either as single additive or as part of a chemical mixture, facilitated nicotine uptake. Furthermore, the data did not suggest that differences in the inhalation pattern of cigarette smoke of any of the Priority Additives tested occurred when compared to the additive-free reference cigarette. Finally, it is concluded that neither the scientific literature nor our study gave circumstantial indications of increased addictiveness for cigarettes containing these priority additives.” (McEwan et al. 2019).

“Evidence suggests exposure of nicotine-containing e-cigarette aerosol to nonusers leads to systemic absorption of nicotine. However, no studies have examined acute secondhand exposures that occur in public settings. Here, we measured the serum, saliva and urine of nonusers pre- and post-exposure to nicotine via e-cigarette aerosol. Secondarily, we recorded factors affecting the exposure. Six nonusers of nicotine-containing products were exposed to secondhand aerosol from ad libitum e-cigarette use by three e-cigarette users for 2 h during two separate sessions (disposables, tank-style). Pre-exposure (baseline) and post-exposure peak levels (Cmax) of cotinine were measured in nonusers' serum, saliva, and urine over a 6-hour follow-up, plus a saliva sample the following morning. We also measured solution consumption, nicotine concentration, and pH, along with use behavior. Baseline cotinine levels were higher than typical for the US population (median serum session one = 0.089 ng/ml; session two = 0.052 ng/ml). Systemic absorption of nicotine occurred in nonusers with baselines indicative of no/low tobacco exposure, but not in nonusers with elevated baselines. Median changes in cotinine for disposable exposure were 0.007 ng/ml serum, 0.033 ng/ml saliva, and 0.316 ng/mg creatinine in urine. For tank-style exposure they were 0.041 ng/ml serum, 0.060 ng/ml saliva, and 0.948 ng/mg creatinine in urine. Finally, we measured substantial differences in solution nicotine concentrations, pH, use behavior and consumption. Our data show that although exposures may vary considerably, nonusers can systemically absorb nicotine following acute exposure to secondhand e-cigarette aerosol. This can particularly affect sensitive subpopulations, such as children and women of reproductive age.” (Melstrom et al. 2018).

“Nicotine is rapidly absorbed through the oral cavity, lung, skin, urinary bladder and gastrointestinal tract. The rate of nicotine absorption through the biological membranes is a pH dependent process (Schevelbein et al., 1973). In its ionized state, such as in the stomach, nicotine does not rapidly cross membranes but it is well absorbed in the small intestine, which has a more alkaline pH and a large surface. The respiratory absorption of nicotine was found to be 60-80% (DAR, 2008). Following the administration of nicotine capsules or nicotine in solution, peak concentrations in blood are reached in about 1 hour (Benowitz et al., 1991; Zins et al., 1997; Dempsey et al., 2004). The oral bioavailability of nicotine is incomplete because of hepatic first-pass metabolism, and usually ranges between 20% to 45%” (Benowitz et al., 1991).

“It has been demonstrated that nicotine is mainly secreted through urine and faces but also in bile, gastric juice, sweat (Perlman et al., 1942; Fishman, 1963; Seaton et al., 1993). The rate of nicotine excretion is influenced by the pH of the urine. When the pH is alkaline, the proportion of uncharged nicotine increases and re-absorption occurs, as a result, less nicotine is excreted” (Becket et al., 1965).

“Pharmacokinetics and delivery of nicotine after single use of a heated tobacco product were generally comparable with smoking a cigarette. However, studies that compared ad libitum use of heated tobacco products with smoking cigarettes consistently reported lower nicotine levels in heated tobacco product users compared with smokers. Probably to compensate, smokers who were switched to using heated tobacco products adjusted their puffing behaviour.

All forms of NRT deliver nicotine much more slowly and at lower doses than smoking, but the speed and amount vary according to the delivery system (oral, dermal or nasal) and the dose. The faster acting NRT products (nasal and mouth spray) deliver peak plasma nicotine levels within about 10 minutes. Use of NRT therefore results in much slower nicotine delivery than smoking. Absorption of nicotine is also affected by other factors such as pH.

Similar to oral nicotine products, nicotine absorption with snus also occurs through the buccal route, aided by the alkaline pH of snus. As such, nicotine absorption is slower when using snus, than from cigarette smoking, as there are no arterial boli of nicotine delivered to the brain. Nevertheless, nicotine exposure overall can be very similar between snus users and cigarette smokers.

Experienced users can achieve greater increases in blood nicotine levels than naïve users under the same puffing regime, albeit slower than from cigarette smoking (75-77). Studies with experienced users found comparable or, in some cases, higher venous blood nicotine levels than with cigarette smokers. A study with 16 experienced users and high nicotine concentrations (36mg/mL) found a higher pre-post nicotine boost following a standardised puffing regimen than that typically seen with tobacco cigarettes (78). A further study (77) with 30 participants (10 smokers and 20 experienced EC users) found similar doses and speed of nicotine delivery to tobacco cigarettes among those using third generation devices (mods). An additional study, with 13 experienced users again during a standardised puffing session demonstrated that venous nicotine blood levels of experienced EC users from later generation devices were comparable to, and in some cases higher than those of smokers (79). Most of the participants had peak nicotine levels within two to five minutes after puffing an EC, suggestive of pulmonary delivery and likely to lead to dependence, although not all the nicotine retained was absorbed through the lungs (79). The same study also included a subsequent ad libitum phase (80). Here the authors found that vaping behaviour differed from smoking behaviour in that EC users took longer puffs and grouped their puffs in shorter clusters (two to five puffs). The intermittent puffing patterns led to a more gradual rise in plasma nicotine levels across the session, in contrast to the bolus dosing from cigarette smoking. Nicotine intake was related to puff topography only for the tank users but not across the whole sample (which included cigalikes and other devices)” (Public Health England, 2018).

“Knowledge about the change in blood nicotine concentrations during the first five minutes (acute phase) of e-cigarette vaping is important to determine whether the used product has a dependence potential or may be an efficient nicotine replacement product. To address this issue, we monitored blood nicotine levels during the acute phase in volunteers using disposable cigalikes (CLs) and a tank model (TM) and compared them with blood nicotine levels in subjects using a tobacco cigarette (TC). In parallel, heart rate changes were continually measured and withdrawal symptoms and craving were assessed with the Questionnaire on Smoking Urges before and immediately after the vaping/smoking sessions. Additionally, at the end of each session negative health effects were rated on a visual analog scale. After five minutes of e-cigarette or TC use, the mean nicotine plasma concentrations were as follows: CLs, 5.5ng/ml; TM, 9.3ng/ml; TC, 17.1ng/ml. Nicotine levels increased significantly faster in the first 4min of consuming a TC than with the CLs and the TM. The highest rate of increase in nicotine concentration was found with the TC (6.8ng/ml) and TM (2.3ng/ml) between the 1st and 2nd minute, whereas the CLs showed comparatively small changes in the amount delivered over the five minutes. Withdrawal and craving for smoking decreased with the TM by the same amount as with the TC, even though less nicotine was delivered to the blood and considerably fewer side effects occurred. The heart rate of TM users was also markedly lower than that of the TC users. Unlike CLs, TM e-cigarettes represent an effective source of nicotine and might be used as an alternative nicotine replacement product to aid smoking cessation. However, nicotine plasma levels observed in TM users after short-time vaping have also the potential to produce and sustain nicotine addiction.”(Rüther et al. 2018)

“Quantitative analysis of antagonism is infrequently used to identify nAChRs mediating behavioral effects. Here, nicotine (0.032 mg/kg i.v.) was established as a discriminative stimulus in rhesus monkeys responding under a fixed ratio 5 schedule; pharmacokinetics and underlying nAChR mechanism(s) were examined. When measured up to 4 h in venous blood, the training dose resulted in the following mean pharmacokinetic parameters: nicotine Cmax = 71.7 ng/ml, t1/2 = 116 min, and clearance = 6.25 ml/min/kg; cotinine Cmax = 191 ng/ml; and 3OH-cotinine Cmax = 63 ng/ml. The ED50 value of nicotine to produce discriminative stimulus effects was 0.013 mg/kg. Epibatidine and varenicline increased drug-lever responding to 97% and 95%, respectively (ED50 values = 0.00015 and 0.031 mg/kg, respectively), whereas cocaine, midazolam, and morphine produced no more than 28% drug-appropriate responding. Mecamylamine and dihydro-β-erythroidine (DHβE) dose-dependently attenuated the discriminative stimulus effects of the nicotine training dose, whereas methyllycaconitine (MLA) did not. DHβE (0.1 and 0.32) produced rightward shifts of the nicotine and varenicline dose-response functions; Schild plots fitted through individual data resulted in slopes that were not different from unity; the apparent pA2 calculated for DHβE did not significantly differ in the presence of nicotine (6.58) or varenicline (6.45). Compared to human cigarette smoking, nicotine blood levels after 0.032 mg/kg nicotine i.v. took a similar time to reach maximal concentration, levels at Cmax were similar to smoking 2-3 cigarettes, while average nicotine levels were comparable to smoking 5-6 cigarettes. Apparent pA2 analysis with DHβE under these conditions is consistent with nicotine and varenicline acting through the same nAChRs to produce discriminative stimulus effects.” (Moerke et al. 2017).

## *4.3.* *Interactions*

“Pregnant smoking women are frequently episodic drinkers. Here, we investigated whether ethanol exposure restricted to the brain growth spurt period when combined with chronic developmental exposure to nicotine aggravates memory/learning deficits and hyperactivity, and associated cAMP and cGMP signaling disruption. To further investigate the role of these signaling cascades, we verified whether vinpocetine (a phosphodiesterase inhibitor) ameliorates the neurochemical and behavioral outcomes. Swiss mice had free access to nicotine (NIC, 50 μg/ml) or water to drink during gestation and until the 8th postnatal day (PN8). Ethanol (ETOH, 5 g/kg, i.p.) or saline were injected in the pups every other day from PN2 to PN8. At PN30, animals either received vinpocetine (20 mg/kg, i.p.) or vehicle before being tested in the step-down passive avoidance or open field. Memory/learning was impaired in NIC, ETOH and NIC + ETOH mice, and vinpocetine mitigated ETOH- and NIC + ETOH-induced deficits. Locomotor hyperactivity identified in ETOH and NIC + ETOH mice was ameliorated by vinpocetine. While cyclic nucleotides levels in cerebral cortex and hippocampus were reduced by NIC, ETOH and NIC + ETOH, this outcome was more consistent in the latter group. As observed for behavior, vinpocetine normalized NIC + ETOH nucleotides levels. pCREB levels were also increased in response to vinpocetine, with stronger effects in the NIC + ETOH group. Exposure to both drugs of abuse worsens behavioral and neurochemical disruption. These findings and the amelioration of deleterious effects by vinpocetine support the idea that cAMP and cGMP signaling contribute to nicotine- and ethanol-induced hyperactivity and memory/learning deficits.” (Abreu-Villaca et al. 2018).

“BACKGROUND: Psychoactive substance abuse is a health problem worldwide. Has been reported a high prevalence of use of tobacco and cocaine, either separately or in combination. Clinical and animal studies have suggested that the concurrent use of cocaine and nicotine reinforces the potency of one or both drugs and that nicotine may enhance the reinforcing effects of cocaine. Our study evaluated the combined effects of cocaine and nicotine on locomotor activity during the induction and expression phases of locomotor sensitization-a physiological mechanism that plays an important role in establishing some of the defining characteristics of drug abuse. METHODS: We used Wistar rats which were dosed with cocaine, nicotine or cocaine and nicotine combination and recorded their locomotor activity in different phases of the experiment. RESULTS: We found that a daily dose of cocaine combined with nicotine enhanced cocaine- and nicotine-induced locomotor activity, as well as induction and expression of locomotor sensitization. Moreover, we found that pretreatment with nicotine enhanced the locomotor sensitization expression. CONCLUSION: These results suggest that concurrent use of cocaine and nicotine may result in co-abuse of these drugs.” (Barbosa-Méndez and Salazar-Juárez 2018).

“Cigarette smokers with brain damage involving the insular cortex display cessation of tobacco smoking, suggesting that this region may contribute to nicotine addiction. In the present study, we speculated that molecules in the insular cortex that are sensitive to experimental traumatic brain injury (TBI) in mice might provide leads to ameliorate nicotine addiction. Using targeted lipidomics, we found that TBI elicited substantial increases of a largely uncharacterized lipid, N-acyl-glycine, N-oleoyl-glycine (OlGly), in the insular cortex of mice. We then evaluated whether intraperitoneal administration of OlGly would alter withdrawal responses in nicotine-dependent mice as well as the rewarding effects of nicotine, as assessed in the conditioned place preference paradigm (CPP). Systemic administration of OlGly reduced mecamylamine-precipitated withdrawal responses in nicotine-dependent mice and prevented nicotine CPP. However, OlGly did not affect morphine CPP, demonstrating a degree of selectivity. Our respective in vitro and in vivo observations that OlGly activated peroxisome proliferator-activated receptor alpha (PPAR-α) and the PPAR-α antagonist GW6471 prevented the OlGly-induced reduction of nicotine CPP in mice suggests that this lipid acts as a functional PPAR-α agonist to attenuate nicotine reward. These findings raise the possibility that the long chain fatty acid amide OlGly may possess efficacy in treating nicotine addiction.” (Donvito et al. 2018).

“BACKGROUND AND OBJECTIVE: The aim of this study was to test the hypothesis that tincture of benzoin (TOB) facilitates immediate transmucosal nicotine absorption while simultaneously promoting a safe and sustained delivery of the nicotine. METHODS: In combination with TOB, nicotine toxicity and diffusion across human mucosal cells were measured using a 3-D human mucosal tissue model. RESULTS: Nicotine was delivered 2.1 times more quickly in combination with TOB than in combination with saline (p < 0.05). Despite the increased diffusion, nicotine in combination with TOB significantly increased mucosal cell survival (p < 0.05) by reducing the release of mitochondrial cytochrome c into the cytoplasm when compared with nicotine without TOB. The average percentage distribution of cytochrome c in the cytosolic fraction over time of nicotine + 79% ethyl alcohol (ETOH) versus nicotine plus TOB (79% ETOH) was significantly different over 120 min (60.0 ± 29.9% cytosol, 16.1 ± 9.4% cytosol, p = 0.03). Related to the reduction of cytochrome c release into the cytoplasm, TOB suppressed caspase-3 and -9 activity, thereby preventing intrinsic apoptosis and providing cytoprotection of the mucosal cells (ETOH + nicotine vs ETOH + nicotine + TOB: p = 0.008 for caspase 3, p < 0.001 for caspase 9). CONCLUSION: Two hours of TOB (17-24% benzoin, 79% ETOH) plus nicotine promotes diffusion of nicotine across human mucosal cells and simultaneously prevents human mucosal cell toxicity by inhibiting cytochrome c release into the cytosol, thereby preventing caspase 3 and 9 activity and subsequent intrinsic apoptosis” (Battaglia and Nguyen 2017).

“BACKGROUND/AIM: We have shown that either chronic nicotine (NIC) exposure or 5-aza-cytidine (AZA) augments oxidative stress-dependent injury through stimulating p66shc in renal cells. Hence, NIC could exacerbate adverse effects of AZA while antioxidants such as resveratrol (RES) could prevent it. MATERIALS AND METHODS: Renal proximal tubule cells (NRK52E) were treated with 20 μM RES prior to 200 μM NIC plus 100 nM AZA and cell injury (LDH release) was determined. Reporter luciferase assays determined p66shc activation and RES-induced antioxidant responses. Genetic manipulations identified the mechanism of RES action. RESULTS: NIC exacerbated AZA-dependent injury via augmenting p66shc transcription. While RES suppressed NIC+AZA-mediated injury, -surprisingly-it further enhanced activity of the p66shc promoter. RES protected cells via the cytoplasmic p66shc/Nrf2/heme oxygenase-1 (HO-1) axis. CONCLUSION: RES can protect the kidney from adverse effects of NIC in patients undergoing anticancer therapy.”(Arany et al. 2017).

“BACKGROUND: Nicotine is a major toxic component of tobacco smoke and has been recognized as a risk factor to induce oxidative tissue damage, which is a precursor to cardiovascular diseases, lung-related diseases, and cancers. Peaches (*Prunus persica*) have been used for the treatment of degenerative disorders, such as hypermenorrhea, dysmenorrhea, and infertility in Asian countries. In this study, we investigated the effects of white-fleshed peach on the excretion of nicotine metabolites and 1-hydroxypyrene in smokers and chronic nicotine-induced tissue damages in mice. METHODS: The concentrations of cotinine and 1-hydroxypyrene were measured in urine of smokers before or after intake of white-fleshed peaches. In addition, ICR mice were injected with nicotine (5 mg/kg body weight) and then orally administered with white-fleshed peach extracts (WFPE) (250 or 500 mg/kg body weight) for 36 days. The oxidative stress parameters and the activities of antioxidant enzymes were measured in liver and kidney tissues. Also, histological changes and nitrotyrosine expression were assessed. RESULTS: Intake of white-fleshed peaches increased the urinary concentration of nicotine metabolites and 1-hydroxypyrene in 91.67% and 83.33% of smokers, respectively. WFPE decreased the malondialdehyde levels and recovered the activities of antioxidant enzymes in nicotine-injected mice. In addition, WFPE inhibited nitrotyrosine expression and inflammatory responses in the liver, kidney, and lung tissues of nicotine-treated mice. CONCLUSIONS: White-fleshed peaches may increase the metabolism of toxic components in tobacco smoke in smokers and protect normal tissues against nicotine toxicity in mice. Therefore, supplementation of white-fleshed peaches might be beneficial to smokers.” (Kim et al. 2017).

“Nicotine exposure during pregnancy induces oxidative stress and leads to behavioral alterations in early childhood and young adulthood. The current study aimed to investigate the possible protective effects of green tea (*Camellia sinensis*) against perinatal nicotine-induced behavioral alterations and oxidative stress in mice newborns. Pregnant mice received 50 mg/kg *C. sinensis* on gestational day 1 (PD1) to postnatal day 15 (D15) and were subcutaneously injected with 0.25 mg/kg nicotine from PD12 to D15. Nicotine-exposed newborns showed significant delay in eye opening and hair appearance and declined body weight at birth and at D21. Nicotine induced neuromotor alterations in both male and female newborns evidenced by the suppressed righting, rotating, and cliff avoidance reflexes. Nicotine-exposed newborns exhibited declined memory, learning, and equilibrium capabilities, as well as marked anxiety behavior. *C. sinensis* significantly improved the physical development, neuromotor maturation, and behavioral performance in nicotine-exposed male and female newborns. In addition, *C. sinensis* prevented nicotine-induced tissue injury and lipid peroxidation and enhanced antioxidant defenses in the cerebellum and medulla oblongata of male and female newborns. In conclusion, this study shows that *C. sinensis* confers protective effects against perinatal nicotine-induced neurobehavioral alterations, tissue injury, and oxidative stress in mice newborns.” (Ajarem et al. 2017).

“The adverse effects of prenatal nicotine and alcohol exposure on human reproductive outcomes are a major scientific and public health concern. In the United States, substantial percentage of women (20-25%) of childbearing age currently smoke cigarettes and consume alcohol, and only a small percentage of these individuals quit after learning of their pregnancy. However, there are very few scientific reports on the effect of nicotine in prenatal alcohol exposure on the cerebellum of the offspring. Therefore, this study was conducted to investigate the cerebellar neurotoxic effects of nicotine in a rodent model of Fetal Alcohol Spectrum Disorder (FASD). In this study, we evaluated the behavioral changes, biochemical markers of oxidative stress and apoptosis, mitochondrial functions and the molecular mechanisms associated with nicotine in prenatal alcohol exposure on the cerebellum. Prenatal nicotine and alcohol exposure induced oxidative stress, did not affect the mitochondrial functions, increased the monoamine oxidase activity, increased caspase expression and decreased ILK, PSD-95 and GLUR1 expression without affecting the GSK-3β. Thus, our current study of prenatal alcohol and nicotine exposure on cerebellar neurotoxicity may lead to new scientific perceptions and novel and suitable therapeutic actions in the future.” (Bhattacharya et al. 2018).

“While the toxicity of the main constituents of electronic cigarette (ECIG) liquids, nicotine, propylene glycol (PG), and vegetable glycerin (VG), has been assessed individually in separate studies, limited data on the inhalation toxicity of them is available when in mixtures. In this 90-day subchronic inhalation study, Sprague-Dawley rats were nose-only exposed to filtered air, nebulized vehicle (saline), or three concentrations of PG/VG mixtures, with and without nicotine. Standard toxicological endpoints were complemented by molecular analyses using transcriptomics, proteomics, and lipidomics. Compared with vehicle exposure, the PG/VG aerosols showed only very limited biological effects with no signs of toxicity. Addition of nicotine to the PG/VG aerosols resulted in effects in line with nicotine effects observed in previous studies, including up-regulation of xenobiotic enzymes (Cyp1a1/Fmo3) in the lung and metabolic effects, such as reduced serum lipid concentrations and expression changes of hepatic metabolic enzymes. No toxicologically relevant effects of PG/VG aerosols (up to 1.520 mg PG/L + 1.890 mg VG/L) were observed, and no adverse effects for PG/VG/nicotine were observed up to 438/544/6.6 mg/kg/day. This study demonstrates how complementary systems toxicology analyses can reveal, even in the absence of observable adverse effects, subtoxic and adaptive responses to pharmacologically active compounds such as nicotine.” (Phillips et al. 2017)

“Studies in animal models have suggested that nicotine, an agonist of nicotinic acetylcholine receptors (nAChRs), may have the potential to prevent and/or reverse the peripheral neuropathy induced by cancer chemotherapeutic drugs, such as paclitaxel and oxaliplatin. However, a large body of evidence suggests that nicotine may also stimulate lung tumor growth and/or interfere with the effectiveness of cancer chemotherapy. While the reported proliferative effects of nicotine are highly variable, the antagonism of antitumor drug efficacy is more consistent, although this latter effect has been demonstrated primarily in cell culture studies. In contrast, in vitro and in vivo studies from our own laboratory indicate that nicotine fails to enhance the growth of non-small cell lung cancer cells or attenuate the effects of chemotherapy (paclitaxel). Given the inconsistencies in the literature, coupled with our own findings, the weight of evidence suggests that caution may be warranted in proposing to utilize nicotine to mitigate chemotherapy-induced peripheral neuropathy in cancer patients receiving chemotherapy. Conversely, clinical trials could be performed in patients who have completed therapy and are considered to be disease-free to determine whether nicotine, in the form of commercially available patches or gum, is effective in alleviating peripheral neuropathy symptoms.” (Kyte and Gewirtz 2018).

“Increased risk of attention-deficit/hyperactivity disorder (AD/HD) is partly associated with the early developmental exposure to nicotine in tobacco smoke. Emerging reports link tobacco smoke exposure or prenatal nicotine exposure (PNE) with AD/HD-like behaviors in rodent models. We have previously reported that PNE induces cognitive behavioral deficits in offspring and decreases the contents of dopamine (DA) and its turnover in the prefrontal cortex (PFC) of offspring It is well known that the dysfunction of DAergic system in the brain is one of the core factors in the pathophysiology of AD/HD. Therefore, we examined whether the effects of PNE on the DAergic system underlie the AD/HD-related behavioral changes in mouse offspring. PNE reduced the release of DA in the medial PFC (mPFC) in mouse offspring. PNE reduced the number of tyrosine hydroxylase (TH)-positive varicosities in the mPFC and in the core as well as the shell of nucleus accumbens, but not in the striatum. PNE also induced behavioral deficits in cliff avoidance, object-based attention, and sensorimotor gating in offspring. These behavioral deficits were attenuated by acute treatment with atomoxetine (3 mg/kg, s.c.) or partially attenuated by acute treatment with MPH (1 mg/kg, s.c.). Taken together, our findings support the notion that PNE induces neurobehavioral abnormalities in mouse offspring by disrupting the DAergic system and improve our understanding about the incidence of AD/HD in children whose mothers were exposed to nicotine during their pregnancy.” (Alkam et al. 2017).

“AIM: Nicotine exerts a number of physiological effects. The purpose of this study was to determine the effects of nicotine on thioacetamide (TAA)-induced liver fibrosis in mice. MATERIALS AND METHODS: For in vivo experiments, hepatic fibrosis was induced by TAA (0.25 g/kg, i.p.) three times a week for 6 weeks. Mice of TAA treated groups were administered daily with distilled water and nicotine (50 or 100 μg/mL) via gastrogavage throughout the experimental period. For in vitro experiments, HepG2 (human liver cancer cell line) and LX-2 (human hepatic stellate cell line) were used to determine oxidative stress and fibrosis, respectively. RESULTS: Compared to control groups, TAA treated groups had significantly differences in serum alanine transferase and aspartate aminotransferase levels and nicotine accentuated liver injury. Moreover, nicotine increased the mRNA levels of TAA-induced transforming growth factor-β (TGF-β) and collagen type I alpha 1 in the liver. Nicotine also increased TAA-induced oxidative stress. Histological examination confirmed that nicotine aggravated the degree of fibrosis caused by TAA treatment. Additionally, nicotine enhanced hepatic stellate cell activation via promoting the expression of α-smooth muscle actin. CONCLUSIONS: Oral administration of nicotine significantly aggravated TAA-induced hepatic fibrosis in mice through enhancing TGF-β secretion and TAA-induced oxidative stress. The increase in TGF-β levels might be associated with the strengthening of oxidative processes, subsequently leading to increased hepatic stellate cell activation and extracellular matrix deposition. These results suggest that patients with liver disease should be advised to abandon smoking since nicotine may exacerbate hepatic fibrosis.” (Zhou Z et al. 2017).

# *5.* *Toxicity*

## *5.1.* *Single dose toxicity*

“Electronic cigarettes (ECs) are a device that aerosolize liquid nicotine by heating a solution of nicotine, glycerol and flavoring agents. The awareness and the usage of ECs has increased in many countries. Due to the online sales and the absence of EC regulations, the prevalence of EC usage is especially high in adolescents and young adults. Due to the large amount and the high nicotine concentration of EC liquid, the ingestion for suicide can lead to cardiac death. We had two patients, a 27-year-old male who ingested about 23 mg/kg of nicotine and a 17-year-old female who ingested about 30 mg/kg of nicotine. Both patients presented seizure-like movement and cardiac arrest. They had metabolic acidosis and transient cardiomyopathy. They were ultimately discharged with a cerebral performance category of 2 and 4, respectively. Increasing EC use may produce more cases of medical problems or suicide by nicotine intoxication.” (Park et al. 2018).

“Nicotine is a dangerous substance extracted from tobacco leaves. When nicotine is absorbed in excessive amounts, it can lead to respiratory failure and cardiac arrest. The commercialization of electronic cigarettes (e-cigarettes) has allowed users to directly handle e-cigarette liquid. Consequently, the risk of liquid nicotine exposure has increased. We describe our experience of managing the case of a patient who orally ingested a high concentration of liquid nicotine from e-cigarette liquid. The patient presented with bradycardia and hypotension, which are symptoms of parasympathetic stimulation, together with impaired consciousness. He recovered following treatment with atropine and a vasopressor.” (Paik et al. 2018).

“Nicotine exerts it effects via stimulation of the nicotinic acetylcholine receptors located in the central nervous system and target organs throughout the body as part of the parasympathetic autonomic nervous system (USDHHS, 2010). As a result of the global expression of these receptors, their stimulation causes broad physiologic effects.

Acute toxicological data on nicotine is limited. Such information comes from three sources (1) animal studies, (2) studies investigating nicotine as a therapeutic agent (including NRT), (3) poisoning involving nicotine.

The lethal dose of nicotine has been estimated to be 40–60 mg (0.6 to 1.0 mg/kg bw) for adults (Gosselin, 1988), and about 10 mg for children (Arena, 1974). Persons have widely different levels of tolerance to the toxic effects of nicotine. Apart from local caustic action, the target organs are the peripheral and central nervous systems. Poisoning in man is associated with a burning sensation in the mouth and throat, salivation, nausea, abdominal pain, vomiting and diarrhoea. Gastrointestinal reactions are less severe but can occur even after dermal and respiratory exposure. Systemic effects include: agitation, headache, sweating, dizziness, auditory and visual disturbances, confusion, weakness and lack of coordination. A transient increase in blood pressure, followed by hypertension, bradycardia, paroxysmal atrial fibrillation, or cardiac standstill may be observed. In severe poisoning, tremor, convulsions and coma occur. Faintness, prostration, cyanosis and dyspnea progress to collapse. Death may occur from paralysis of respiratory muscles and/or central respiratory failure.” (EFSA, 2009).

“Studies investigating nicotine as a therapeutic agent in humans are limited in predicting its acute toxicity. These studies document better the adverse effects rather than overt toxicity, as the doses administered are chosen are considered subtoxic. Mild adverse effects of nicotine given as pharmacologic treatment comprise of epigastric pain, headache, myalgia, sleep disturbance, irritability, pruritus and rash.” (Barrueco et al., 2005; Lunney and Leong, 2012).

Numerous poisonings have been documented in the literature since the use of nicotine as a pesticide became widespread in early twentieth century, as well the increasing use of e-cigarettes. These case studies describe patients exposed to doses associated with toxicity via one or more routes of exposure. Despite the abundance of case reports, it appears that there has not been a systematic assessment of the literature to characterize the dose response relationship.

“In the series of pediatric patients, unintentional and brief (20 minutes or less) exposures to used TNPs resulted in few, if any medical symptoms. Children with dermal exposures of longer duration were more likely to become symptomatic than children who had bitten or sucked briefly on a patch. Even with higher absorbed nicotine doses, most children experienced only mild gastrointestinal symptoms or skin irritation; however, some children with dermal exposures to new or used TNPs for longer than 60 minutes (a calculated dose of absorbed nicotine was 0.9 mg) manifested typical symptoms of nicotine poisoning including gastrointestinal distress (nausea, vomiting, diarrhea, abdominal pain), weakness and dizziness.” (Woolf et al., 1997).

Christensen LB et al., (2013) describes 3 cases of suicide attempts by ingestion of e-cigarette nicotine liquid. Case 1: a woman, age 36, had ingested 20 mL of nicotine liquid labelled as containing 18 mg. The Poisons Information Center was contacted 10 minutes after the intake. The patient presented no symptoms. Case 2: The same woman was admitted to the emergency after ingestion of 50 mL of nicotine liquid labelled as containing 30 mg of nicotine/mL i.e., 1500 mg of nicotine in total. Two hours after ingestion the symptoms present were abdominal pain, nausea, and voluminous vomiting, the patient was received the activated charcoal and kept for the observation for 6 hours. Case 3: Male, age 13, ingested 3 mL of nicotine liquid, no information about concentration. He was treated with activated charcoal and 1 hour after ingestion symptoms were decreasing.

Eberlein et al., (2014) reported a case of a 24-year-old German man with a depressive disorder who was admitted to the emergency ward after trying to poison himself by ingesting one capsule of the nicotine liquid used in electronic cigarettes, containing 180 mg of nicotine. Immediately after ingestion, he called an ambulance and reached the hospital within 30 minutes. The patient reported that about 10 minutes after ingestion he started vomiting. Nausea continued for a few hours, but no more vomiting was observed. Patient received 3000 mg of charcoal. Within the first hour of treatment, his reported dizziness decreased. Initial blood pressure measurements indicated a slight hypertension, and tachycardic heart rates were observed. Both symptoms vanished within the next 3 hours.

“34-year-old male smoker, has been admitted to the hospital after voluntarily ingesting liquid from nine e-cigarette cartridges, each containing 160 mg of nicotine (total of 1440 mg nicotine). Man also consumed 0.5 L of vodka. Several minutes after the e-liquid ingestion, the man lost his consciousness and went into a sudden cardiac arrest. During the resuscitation process, patient received 3 mg of atropine (among others) and his basic vital functions returned to normal, however, he remained in coma for 5-6 hours. After several hours of normalization period, the neurological examination did not reveal greater deviations from the norm. The day following the admission, patient left hospital in good general health” (Waldman and Sein Anand, 2012).

“A 22-year-old male was admitted to the emergency department (ED) shortly after intentional ingestion of approximately 30 ml of e-liquid (nicotine content was 24 mg/ml). 20 minutes after ingestion, he developed nausea and vomiting. He then rubbed an additional 30 ml over his skin. His family contacted emergency medical services (EMS), who performed skin decontamination prior to arrival to the ED. In the ED, the patient complained of dizziness and nausea, with a heart rate ranging from 51-58 bmp. A mild tremor was noted during physical exam. Though bradycardia and nausea persisted for several hours, the patient completely recovered with the supportive measures.” (Valento, 2013).

“A previously healthy 27-year-old man with a borderline personality disorder was admitted to the emergency department (ED) one hour after ingestion of five e-liquid fillings in a suicide attempt. He also had consumed five units of wine. The e-liquid fillings contained a total of 420 mg of nicotine and unknown amounts of propylene glycol and glycerin. Before arrival at the ED the patient had vomited three times, but upon arrival he was free of complaints. The patient denied ingestion of other substances. Initial evaluation showed tachycardia, high blood pressure and normal oxygen saturation. Apart from excessive salivation, his physical examination was unremarkable. Laboratory examination revealed a high anion and osmol gap. Patient received the activated charcoal twice and during observational period of 30 hours no other adverse effects occurred. The metabolic acidosis disappeared within 10 hours after admission.” (Schipper et al. 2014).

Recently, Mayer B (2014) managed to trace the statement concerning the low lethal toxicity values of nicotine back to self-experiment in the 19th century. Mayer concluded “Nicotine is a toxic compound that should be handled with care, but the frequent warnings of potential fatalities caused by ingestion of small amounts of tobacco products or diluted nicotine-containing solutions are unjustified and need to be revised in light of overwhelming data indicating that more than 0.5 g (500 mg) of oral nicotine is required to kill an adult”.

In animals as in humans, nicotine is acutely toxic by all routes of exposure. It causes initial hyperexcitability, hyperpnea, salivation, vomiting, and diarrhea and then depression, incoordination, and paralysis in both small and large animals.

The reported oral LD50 values for laboratory rats range from 50 to 188 mg/kg (Sine, 1993 cited in NIOSH; Klaassen CD, 2013; DECOS 2004). The oral LD50 values for mice range from 3.34 to 24 mg/kg (Lazutka et al., 1969; Trochimowicz et al., 1994; DECOS, 2004). The dermal LD50 for rabbits and rat were reported 50 and 140 mg/kg, respectively (Trochimowicz et al., 1994).

No LC50 values were reported for inhalation route. One study investigated the role of nicotine in the development of pulmonary emphysema in Long-Evans rats. Animals (n=13-16) received single intratracheally administered dose of nicotine, 3 or 7.5 mg/kg bw. Four weeks after treatment, ventilator, mechanical and gas exchange functions were not significantly different compared with control rats. It was concluded that the intratracheal installation of a single, relatively high dose of nicotine, did not induce the development of pulmonary emphysema in the rat (Kimmel and Diamond, 1984).

Acute toxicity of nicotine was examined in old (24 months) and young (6 weeks) Wistar rats. There were no significant age-related differences in the mortality and convulsive responses induced by an intraperitoneal injection of 24.5 mg/kg of nicotine. Cortical and blood nicotine levels at the time of death and 15 minutes after nicotine injection were higher in old rats. Additionally, significant age-related decreases in P450 levels, activities of nicotine oxidase and flavin-containing monooxygenase were observed, indicating that the decrease in hepatic metabolism in old rats could be a cause of the increase of nicotine levels in the brain and blood. Moreover, it has been reported that the latent period to death was longer in old rats than in young rats, indicating decrease of brain sensitivity in older animals (Okamoto et al. 1994).

“As we detailed in our last report, the source of the oft-repeated claim about ingestion of 30-60mg of nicotine being fatal, was hard to locate. A recent study (91) concluded that the lower dose limit for fatal nicotine is thought to be considerably higher, in the region of 500-1,000 mg ingested nicotine.” (Public Health England, 2018)

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| --- | --- | --- | --- | --- | --- |
| **Organism** | **Test Type** | **Route** | **Reported Dose (Normalized Dose)** | **Effect** | **Source** |
| cat | LD50 | intravenous | 1300ug/kg (1.3mg/kg) |  | Proceedings of the Society for Experimental Biology and Medicine. Vol. 35, Pg. 316, 1936. |
| cat | LDLo | intramuscular | 9mg/kg (9mg/kg) | PERIPHERAL NERVE AND SENSATION: FLACCID PARALYSIS WITHOUT ANESTHESIA (USUALLY NEUROMUSCULAR BLOCKAGE)  BEHAVIORAL: ATAXIA  BEHAVIORAL: CONVULSIONS OR EFFECT ON SEIZURE THRESHOLD | Science. Vol. 127, Pg. 1054, 1958. |
| cat | LDLo | subcutaneous | 20mg/kg (20mg/kg) |  | "Ueber die Wirkung Verschiedener Gifte Auf Vogel, Dissertation," Forchheimer, L., Pharmakologischen Institut der Universitat Wurzburg, Fed. Rep. Ger., 1931Vol. -, Pg. -, 1931. |
| dog | LD50 | intravenous | 5mg/kg (5mg/kg) |  | Journal of Pharmacology and Experimental Therapeutics. Vol. 95, Pg. 506, 1949. |
| dog | LD50 | oral | 9200ug/kg (9.2mg/kg) |  | Proceedings of the Society for Experimental Biology and Medicine. Vol. 29, Pg. 1177, 1932. |
| dog | LDLo | intramuscular | 7700ug/kg (7.7mg/kg) |  | Proceedings of the Society for Experimental Biology and Medicine. Vol. 29, Pg. 1177, 1932. |
| dog | LDLo | parenteral | 5700ug/kg (5.7mg/kg) |  | Proceedings of the Society for Experimental Biology and Medicine. Vol. 29, Pg. 1177, 1932. |
| dog | LDLo | subcutaneous | 20mg/kg (20mg/kg) |  | "Ueber die Wirkung Verschiedener Gifte Auf Vogel, Dissertation," Forchheimer, L., Pharmakologischen Institut der Universitat Wurzburg, Fed. Rep. Ger., 1931Vol. -, Pg. -, 1931. |
| guinea pig | LD50 | intravenous | 4500ug/kg (4.5mg/kg) |  | Arzneimittel-Forschung. Drug Research. Vol. 11, Pg. 1011, 1961. |
| guinea pig | LDLo | intramuscular | 15mg/kg (15mg/kg) | BEHAVIORAL: CONVULSIONS OR EFFECT ON SEIZURE THRESHOLD  BEHAVIORAL: ATAXIA  PERIPHERAL NERVE AND SENSATION: FLACCID PARALYSIS WITHOUT ANESTHESIA (USUALLY NEUROMUSCULAR BLOCKAGE) | Science. Vol. 127, Pg. 1054, 1958. |
| guinea pig | LDLo | intraperitoneal | 15mg/kg (15mg/kg) |  | Journal of Pharmacology and Experimental Therapeutics. Vol. 50, Pg. 93, 1934. |
| guinea pig | LDLo | subcutaneous | 15mg/kg (15mg/kg) | BEHAVIORAL: ATAXIA  BEHAVIORAL: CONVULSIONS OR EFFECT ON SEIZURE THRESHOLD  LUNGS, THORAX, OR RESPIRATION: DYSPNEA | Journal of Pharmacology and Experimental Therapeutics. Vol. 48, Pg. 95, 1933. |
| horse/donkey | LDLo | intramuscular | 8800ug/kg (8.8mg/kg) | BEHAVIORAL: CHANGES IN MOTOR ACTIVITY (SPECIFIC ASSAY)  BEHAVIORAL: ATAXIA  BEHAVIORAL: SOMNOLENCE (GENERAL DEPRESSED ACTIVITY) | Science. Vol. 127, Pg. 1054, 1958. |
| horse/donkey | LDLo | intramuscular | 8800mg/kg (8800mg/kg) | BEHAVIORAL: CHANGES IN MOTOR ACTIVITY (SPECIFIC ASSAY)  BEHAVIORAL: SOMNOLENCE (GENERAL DEPRESSED ACTIVITY)  BEHAVIORAL: ATAXIA | Science. Vol. 127, Pg. 1054, 1958. |
| human | TDLo | rectal | 1430ug/kg (1.43mg/kg) | GASTROINTESTINAL: NAUSEA OR VOMITING  BEHAVIORAL: "HALLUCINATIONS, DISTORTED PERCEPTIONS" | Clinical Toxicology. Vol. 10, Pg. 391, 1977. |
| mammal (species unspecified) | LD50 | intravenous | 8mg/kg (8mg/kg) |  | "Chemistry of Pesticides," Melnikov, N.N., New York, Springer-Verlag New York, Inc., 1971Vol. -, Pg. 406, 1971. |
| man | LDLo | unreported | 882ug/kg (0.882mg/kg) |  | "Poisoning; Toxicology, Symptoms, Treatments," 2nd ed., Arena, J.M., Springfield, IL, C.C. Thomas, 1970Vol. 2, Pg. 73, 1970. |
| monkey | LDLo | intramuscular | 6mg/kg (6mg/kg) | PERIPHERAL NERVE AND SENSATION: FLACCID PARALYSIS WITHOUT ANESTHESIA (USUALLY NEUROMUSCULAR BLOCKAGE)  BEHAVIORAL: CONVULSIONS OR EFFECT ON SEIZURE THRESHOLD  BEHAVIORAL: ATAXIA | Science. Vol. 127, Pg. 1054, 1958. |
| mouse | LD50 | intraperitoneal | 5900ug/kg (5.9mg/kg) |  | Toxicology and Applied Pharmacology. Vol. 28, Pg. 227, 1974. |
| mouse | LD50 | intravenous | 300ug/kg (0.3mg/kg) | LUNGS, THORAX, OR RESPIRATION: DYSPNEA  BEHAVIORAL: TREMOR  BEHAVIORAL: CONVULSIONS OR EFFECT ON SEIZURE THRESHOLD | British Journal of Pharmacology. Vol. 35, Pg. 161, 1969. |
| mouse | LD50 | oral | 3340ug/kg (3.34mg/kg) | LUNGS, THORAX, OR RESPIRATION: DYSPNEA  BEHAVIORAL: MUSCLE CONTRACTION OR SPASTICITY)  BEHAVIORAL: TREMOR | Hygiene and Sanitation Vol. 34(4-6), Pg. 187, 1969. |
| mouse | LD50 | subcutaneous | 16mg/kg (16mg/kg) |  | Naunyn-Schmiedeberg's Archiv fuer Experimentelle Pathologie und Pharmakologie. Vol. 188, Pg. 605, 1938. |
| mouse | LD50 | unreported | 260ug/kg (0.26mg/kg) |  | European Patent Application. Vol. #0000106, |
| mouse | LDLo | intramuscular | 8mg/kg (8mg/kg) | PERIPHERAL NERVE AND SENSATION: FLACCID PARALYSIS WITHOUT ANESTHESIA (USUALLY NEUROMUSCULAR BLOCKAGE)  BEHAVIORAL: ATAXIA  BEHAVIORAL: CONVULSIONS OR EFFECT ON SEIZURE THRESHOLD | Science. Vol. 127, Pg. 1054, 1958. |
| pig | LDLo | intramuscular | > 14mg/kg (14mg/kg) | BEHAVIORAL: EXCITEMENT  BEHAVIORAL: CHANGES IN MOTOR ACTIVITY (SPECIFIC ASSAY)  BEHAVIORAL: "HALLUCINATIONS, DISTORTED PERCEPTIONS" | Science. Vol. 127, Pg. 1054, 1958. |
| rabbit | LD50 | intraperitoneal | 14mg/kg (14mg/kg) |  | Proceedings of the Society for Experimental Biology and Medicine. Vol. 58, Pg. 231, 1945. |
| rabbit | LD50 | intravenous | 6250ug/kg (6.25mg/kg) |  | Proceedings of the Society for Experimental Biology and Medicine. Vol. 58, Pg. 231, 1945. |
| rabbit | LD50 | skin | 50mg/kg (50mg/kg) | LUNGS, THORAX, OR RESPIRATION: RESPIRATORY DEPRESSION  BEHAVIORAL: CONVULSIONS OR EFFECT ON SEIZURE THRESHOLD | Quarterly Bulletin--Association of Food and Drug Officials of the United States. Vol. 16, Pg. 3, 1952. |
| rabbit | LDLo | intramuscular | 30mg/kg (30mg/kg) | BEHAVIORAL: CONVULSIONS OR EFFECT ON SEIZURE THRESHOLD  BEHAVIORAL: ATAXIA  PERIPHERAL NERVE AND SENSATION: FLACCID PARALYSIS WITHOUT ANESTHESIA (USUALLY NEUROMUSCULAR BLOCKAGE) | Science. Vol. 127, Pg. 1054, 1958. |
| rabbit | LDLo | subcutaneous | 5mg/kg (5mg/kg) |  | "Ueber die Wirkung Verschiedener Gifte Auf Vogel, Dissertation," Forchheimer, L., Pharmakologischen Institut der Universitat Wurzburg, Fed. Rep. Ger., 1931Vol. -, Pg. -, 1931. |
| rat | LD50 | intraperitoneal | 14560ug/kg (14.56mg/kg) |  | Journal of Pharmacology and Experimental Therapeutics. Vol. 124, Pg. 350, 1958. |
| rat | LD50 | intratracheal | 19300ug/kg (19.3mg/kg) |  | American Review of Respiratory Disease. Vol. 129, Pg. 112, 1984. |
| rat | LD50 | intravenous | 2800ug/kg (2.8mg/kg) |  | Drugs in Japan Vol. -, Pg. 916, 1995. |
| rat | LD50 | oral | 50mg/kg (50mg/kg) |  | Farm Chemicals Handbook. Vol. -, Pg. C219, 1991. |
| rat | LD50 | skin | 140mg/kg (140mg/kg) |  | World Review of Pest Control. Vol. 9, Pg. 119, 1970. |
| rat | LD50 | subcutaneous | 25mg/kg (25mg/kg) |  | Farmakologiya i Toksikologiya Vol. 47(5), Pg. 85, 1984. |
| rat | LDLo | intraduodenal | 30mg/kg (30mg/kg) |  | Arzneimittel-Forschung. Drug Research. Vol. 25, Pg. 1037, 1975. |
| rat | LDLo | intramuscular | 15mg/kg (15mg/kg) | BEHAVIORAL: CONVULSIONS OR EFFECT ON SEIZURE THRESHOLD  BEHAVIORAL: ATAXIA  PERIPHERAL NERVE AND SENSATION: FLACCID PARALYSIS WITHOUT ANESTHESIA (USUALLY NEUROMUSCULAR BLOCKAGE) | Science. Vol. 127, Pg. 1054, 1958. |
| rat | LDLo | parenteral | 34mg/kg (34mg/kg) | LUNGS, THORAX, OR RESPIRATION: OTHER CHANGES | Journal of Pharmacology and Experimental Therapeutics. Vol. 48, Pg. 317, 1933. |

As taken from ChemIDplus

Rat - Dermal LD50 – 50 mg/kg bw (BPDB, 2018).

“The fatal human dose has been estimated to be about 50 to 60 mg [Lazutka et al. 1969]. [Note: An oral dose of 50 to 60 mg/kg is equivalent to a 70kg worker being exposed to about 30 to 40 mg/m3 for 30 minutes, assuming a breathing rate of 50 liters per minute and 100% absorption.” (Haz-Map, 2017).

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Spec. Sci. Name**            **Spec. Common Name** | **Media Type** | **Test Loc.** | **Exp. Type** | **Dose #** | **Endpoint** | **Effect** | **Effect Meas.** | **Chem. Anal.** | **Conc. Type**    **Dose** | **Obs. Dur. (Days)** |
| **CAS #/Chemical: 54115 3-[(2S)-1-Methyl-2-pyrrolidinyl]pyridine** | | | | | | | | | | |
| **Mammals** | | | | | | | | | | |
| Mus musculus | NONE | LAB | IP | 6 | ED50 | BEH | GBHV | U | F | 0.017 d |
| House Mouse |  |  |  |  |  |  |  |  | 0.48 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 6 | ED50 | BEH | ATTK | U | F | 0.017 d |
| House Mouse |  |  |  |  |  |  |  |  | 0.49 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 8 | ED50 | BEH | BITE | U | F | 0.024 d |
| House Mouse |  |  |  |  |  |  |  |  | 0.13 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 8 | ED50 | BEH | BITE | U | F | 0.024 d |
| House Mouse |  |  |  |  |  |  |  |  | 0.14 mg/kg |  |
| Mus musculus | NONE | LAB | SC | 2 | LOEL | BCM | HVLA | U | F | 0.076 d |
| House Mouse |  |  |  |  |  |  |  |  | 3 mg/kg |  |
| Mus musculus | NONE | LAB | SC | 2 | LOEL | BCM | HVLA | U | F | 0.076 d |
| House Mouse |  |  |  |  |  |  |  |  | 3 mg/kg |  |
| Mus musculus | NONE | LAB | SC | 2 | LOEL | BCM | 3MTA | U | F | 1 d |
| House Mouse |  |  |  |  |  |  |  |  | 3 mg/kg |  |
| Mus musculus | NONE | LAB | SC | 2 | LOEL | BCM | HVLA | U | F | 1 d |
| House Mouse |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Mus musculus | NONE | LAB | SC | 2 | LOEL | BCM | 3MTA | U | F | 1 d |
| House Mouse |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Mus musculus | NONE | LAB | SC | 2 | LOEL | BCM | 3MTA | U | F | 0.076 d |
| House Mouse |  |  |  |  |  |  |  |  | 3 mg/kg |  |
| Mus musculus | NONE | LAB | SC | 2 | LOEL | BCM | 3MTA | U | F | 0.076 d |
| House Mouse |  |  |  |  |  |  |  |  | 3 mg/kg |  |
| Ovis aries | NONE | LAB | OP | 2 | LOEL | BCM | CO2C | U | F | 0.01 d |
| Domestic Sheep |  |  |  |  |  |  |  |  | 2 mg/ml |  |
| Oryctolagus cuniculus | NONE | LAB | IV | 6 | LOEL | BCM | ARGI | U | F | 0.004 d |
| European Rabbit |  |  |  |  |  |  |  |  | 0.25 mg/kg |  |
| Mus musculus |  | LAB | SC | 2 | LOEL | BCM | HVLA | U | F | 0.042 d |
| House Mouse |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Canis familiaris | NONE | LAB | IV | 2 | LOEL | BCM | PHPH | U | F | 0.021 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 36 ug/kg/mi |  |
| Mus musculus | NONE | LAB | IV | 2 | LOEL | BEH | NMVM | U | F | 1 d |
| House Mouse |  |  |  |  |  |  |  |  | 1 mg/kg/h |  |
| Mus musculus | NONE | LAB | IV | 2 | LOEL | BEH | NMVM | U | F | 1 d |
| House Mouse |  |  |  |  |  |  |  |  | 2 mg/kg/h |  |
| Mus musculus | NONE | LAB | IV | 2 | LOEL | BEH | NMVM | U | F | 1 d |
| House Mouse |  |  |  |  |  |  |  |  | 4 mg/kg/h |  |
| Mus musculus | NONE | LAB | IP | 5 | LOEL | BEH | GBHV | U | F | 0.056 d |
| House Mouse |  |  |  |  |  |  |  |  | 0.1 ug/kg |  |
| Mus musculus | NONE | LAB | SC | 4 | LOEL | BEH | ACTV | U | F | 0.004 d |
| House Mouse |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 4 | LOEL | BEH | VMVT | U | F | 0.005 d |
| House Mouse |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 4 | LOEL | BEH | VMVT | U | F | 0.005 d |
| House Mouse |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 4 | LOEL | BEH | VMVT | U | F | 0.005 d |
| House Mouse |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 4 | LOEL | BEH | VMVT | U | F | 0.005 d |
| House Mouse |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 6 | LOEL | BEH | BITE | U | F | 0.017 d |
| House Mouse |  |  |  |  |  |  |  |  | 0.25 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 8 | LOEL | BEH | BITE | U | F | 0.024 d |
| House Mouse |  |  |  |  |  |  |  |  | 0.50 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 8 | LOEL | BEH | BITE | U | F | 0.024 d |
| House Mouse |  |  |  |  |  |  |  |  | 0.50 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | LOEL | HRM | CORT | U | F | 0.042 d |
| House Mouse |  |  |  |  |  |  |  |  | 5.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | LOEL | HRM | CORT | U | F | 0.104 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | LOEL | HRM | CORT | U | F | 0.104 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | LOEL | HRM | CORT | U | F | 0.104 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | LOEL | HRM | CORT | U | F | 0.104 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | LOEL | HRM | CORT | U | F | 0.104 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | LOEL | HRM | CORT | U | F | 0.104 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | LOEL | HRM | CORT | U | F | 0.021 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.00 mg/kg |  |
| Mus musculus | NONE | LAB | GV | 2 | LOEL | HRM | CORT | U | F | 0.021 d |
| House Mouse |  |  |  |  |  |  |  |  | 4.00 mg/kg |  |
| Ovis aries | NONE | LAB | OP | 2 | LOEL | HRM | NORE | U | F | 0.01 d |
| Domestic Sheep |  |  |  |  |  |  |  |  | 2 mg/ml |  |
| Mesocricetus auratus | NONE | LAB | SC | 2 | LOEL | HRM | GHRM | U | F | 0.021 d |
| Golden Hamster |  |  |  |  |  |  |  |  | 4 mg/kg bdwt |  |
| Mesocricetus auratus | NONE | LAB | SC | 2 | LOEL | HRM | GHRM | U | F | 0.021 d |
| Golden Hamster |  |  |  |  |  |  |  |  | 4 mg/kg bdwt |  |
| Mesocricetus auratus | NONE | LAB | SC | 2 | LOEL | HRM | GHRM | U | F | 0.021 d |
| Golden Hamster |  |  |  |  |  |  |  |  | 4 mg/kg bdwt |  |
| Canis familiaris | NONE | LAB | IV | 2 | LOEL | MPH | SMIX | U | F | 0.005 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.024 mg/kg/min |  |
| Canis familiaris | NONE | LAB | IA | 4 | LOEL | MPH | DMTR | U | F | 0.021 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 10 ug |  |
| Canis familiaris | NONE | LAB | IV | 2 | LOEL | PHY | BLPR | U | F | 0.005 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.024 mg/kg/min |  |
| Mus musculus | NONE | LAB | SC | 2 | LOEL | PHY | BTMP | U | F | 0.076 d |
| House Mouse |  |  |  |  |  |  |  |  | 3 mg/kg |  |
| Mus musculus | NONE | LAB | SC | 2 | LOEL | PHY | BTMP | U | F | 0.076 d |
| House Mouse |  |  |  |  |  |  |  |  | 3 mg/kg |  |
| Canis familiaris | NONE | LAB | IA | 4 | LOEL | PHY | CFLW | U | F | 0.021 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 10 ug |  |
| Mus musculus | NONE | LAB | IP | 2 | LOEL | PHY | EECG | U | F | 0.004 d |
| House Mouse |  |  |  |  |  |  |  |  | 0.62 umol/kg |  |
| Mus musculus | NONE | LAB | IV | 2 | LOEL | PHY | THRG | U | F | 1 d |
| House Mouse |  |  |  |  |  |  |  |  | 1 mg/kg/h |  |
| Mus musculus | NONE | LAB | IV | 2 | LOEL | PHY | THRG | U | F | 1 d |
| House Mouse |  |  |  |  |  |  |  |  | 2 mg/kg/h |  |
| Mus musculus | NONE | LAB | IV | 2 | LOEL | PHY | THRG | U | F | 1 d |
| House Mouse |  |  |  |  |  |  |  |  | 4 mg/kg/h |  |
| Mus musculus | NONE | LAB | IV | 2 | LOEL | PHY | THRG | U | F | 1 d |
| House Mouse |  |  |  |  |  |  |  |  | 4 mg/kg/h |  |
| Mus musculus | NONE | LAB | SC | 2 | LOEL | PHY | THRG | U | F | 1 d |
| House Mouse |  |  |  |  |  |  |  |  | 3 mg/kg |  |
| Mus musculus | NONE | LAB | SC | 2 | LOEL | PHY | THRG | U | F | 1 d |
| House Mouse |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Mus musculus | NONE | LAB | SC | 2 | LOEL | PHY | THRG | U | F | 1 d |
| House Mouse |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 4 | LOEL | PHY | RPRT | U | F | 0.001 d |
| House Mouse |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 4 | LOEL | PHY | RPRT | U | F | 0.001 d |
| House Mouse |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 4 | LOEL | PHY | RPRT | U | F | 0.001 d |
| House Mouse |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 4 | LOEL | PHY | RPRT | U | F | 0.001 d |
| House Mouse |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Ovis aries | NONE | LAB | OP | 2 | LOEL | PHY | GPHY | U | F | 0.01 d |
| Domestic Sheep |  |  |  |  |  |  |  |  | 2 mg/ml |  |
| Ovis aries | NONE | LAB | OP | 2 | LOEL | PHY | GPHY | U | F | 0.01 d |
| Domestic Sheep |  |  |  |  |  |  |  |  | 2 mg/ml |  |
| Canis familiaris | NONE | LAB | IA | 2 | LOEL | PHY | VSCR | U | F | 0.014 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 100 ug |  |
| Canis familiaris | NONE | LAB | IA | 2 | LOEL | PHY | VSCR | U | F | 0.014 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 100 ug |  |
| Canis familiaris | NONE | LAB | OP | 2 | LOEL | PHY | GFRT | U | F | 0.01 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.5 ug/kg/mi |  |
| Canis familiaris | NONE | LAB | OP | 2 | LOEL | PHY | GFRT | U | F | 0.01 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.5 ug/kg/mi |  |
| Canis familiaris | NONE | LAB | IV | 2 | LOEL | PHY | HTRT | U | F | 0.021 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 36 ug/kg/mi |  |
| Canis familiaris | NONE | LAB | OP | 2 | LOEL | PHY | GFRT | U | F | 0.01 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.5 ug/kg/mi |  |
| Canis familiaris | NONE | LAB | OP | 2 | LOEL | PHY | EXCR | U | F | 0.01 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.5 ug/kg/mi |  |
| Canis familiaris | NONE | LAB | OP | 2 | LOEL | PHY | EXCR | U | F | 0.01 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.5 ug/kg/mi |  |
| Canis familiaris | NONE | LAB | OP | 2 | LOEL | PHY | EXCR | U | F | 0.01 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.5 ug/kg/mi |  |
| Canis familiaris | NONE | LAB | OP | 2 | LOEL | PHY | EXCR | U | F | 0.01 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.5 ug/kg/mi |  |
| Canis familiaris | NONE | LAB | OP | 2 | LOEL | PHY | NAEX | U | F | 0.01 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.5 ug/kg/mi |  |
| Canis familiaris | NONE | LAB | OP | 2 | LOEL | PHY | NAEX | U | F | 0.01 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.5 ug/kg/mi |  |
| Canis familiaris | NONE | LAB | OP | 2 | LOEL | PHY | CLEX | U | F | 0.01 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.5 ug/kg/mi |  |
| Canis familiaris | NONE | LAB | OP | 2 | LOEL | PHY | CLEX | U | F | 0.01 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.5 ug/kg/mi |  |
| Mus musculus | NONE | LAB | IP | 2 | NOEL | BCM | WTCO | U | F | 0.014 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.3 mg/kg |  |
| Ovis aries | NONE | LAB | OP | 2 | NOEL | BCM | CO2C | U | F | 0.01 d |
| Domestic Sheep |  |  |  |  |  |  |  |  | 2 mg/ml |  |
| Ovis aries | NONE | LAB | OP | 2 | NOEL | BCM | CO2C | U | F | 0.01 d |
| Domestic Sheep |  |  |  |  |  |  |  |  | 2 mg/ml |  |
| Oryctolagus cuniculus | NONE | LAB | IV | 6 | NOEL | BCM | ARGI | U | F | 0.004 d |
| European Rabbit |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Ovis aries | NONE | LAB | IA | 4 | NOEL | BCM | CO2C | U | F | 0.038 d |
| Domestic Sheep |  |  |  |  |  |  |  |  | 0.5 mg/mi |  |
| Mus musculus | NONE | LAB | IP | 6 | NOEL | BEH | BITE | U | F | 0.017 d |
| House Mouse |  |  |  |  |  |  |  |  | 0.125 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 8 | NOEL | BEH | BITE | U | F | 0.024 d |
| House Mouse |  |  |  |  |  |  |  |  | 0.25 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 8 | NOEL | BEH | BITE | U | F | 0.024 d |
| House Mouse |  |  |  |  |  |  |  |  | 0.25 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 8 | NOEL | BEH | BITE | U | F | 0.024 d |
| House Mouse |  |  |  |  |  |  |  |  | 4.0 mg/kg |  |
| Mus musculus | NONE | LAB | DT | 3 | NOEL | CEL | PCCM | U | A | 1 d |
| House Mouse |  |  |  |  |  |  |  |  | 2 mg/kg bdwt |  |
| Mus musculus | NONE | LAB | IP | 2 | NOEL | GRO | WGHT | U | F | 0.014 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.3 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | NOEL | HRM | CORT | U | F | 0.042 d |
| House Mouse |  |  |  |  |  |  |  |  | 5.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | NOEL | HRM | CORT | U | F | 0.042 d |
| House Mouse |  |  |  |  |  |  |  |  | 5.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | NOEL | HRM | CORT | U | F | 0.042 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | NOEL | HRM | CORT | U | F | 0.042 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | NOEL | HRM | CORT | U | F | 0.042 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | NOEL | HRM | CORT | U | F | 0.052 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | NOEL | HRM | CORT | U | F | 0.052 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | NOEL | HRM | CORT | U | F | 0.052 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | NOEL | HRM | CORT | U | F | 0.042 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | NOEL | HRM | CORT | U | F | 0.042 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | NOEL | HRM | CORT | U | F | 0.042 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | NOEL | HRM | CORT | U | F | 0.052 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | NOEL | HRM | CORT | U | F | 0.052 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | NOEL | HRM | CORT | U | F | 0.052 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | NOEL | HRM | CORT | U | F | 0.021 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.00 mg/kg |  |
| Mus musculus | NONE | LAB | IP | 2 | NOEL | HRM | CORT | U | F | 0.021 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.00 mg/kg |  |
| Mus musculus | NONE | LAB | GV | 2 | NOEL | HRM | CORT | U | F | 0.021 d |
| House Mouse |  |  |  |  |  |  |  |  | 4.00 mg/kg |  |
| Mus musculus | NONE | LAB | GV | 2 | NOEL | HRM | CORT | U | F | 0.042 d |
| House Mouse |  |  |  |  |  |  |  |  | 4.00 mg/kg |  |
| Ovis aries | NONE | LAB | OP | 2 | NOEL | HRM | NORE | U | F | 0.01 d |
| Domestic Sheep |  |  |  |  |  |  |  |  | 2 mg/ml |  |
| Mesocricetus auratus | NONE | LAB | SC | 4 | NOEL | HRM | GHRM | U | F | 0.021 d |
| Golden Hamster |  |  |  |  |  |  |  |  | 7 mg/kg bdwt |  |
| Mus musculus | NONE | LAB | IP | 2 | NOEL | MPH | WGHT | U | F | 0.014 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.3 mg/kg |  |
| Mesocricetus auratus | NONE | LAB | IV | 2 | NOEL | PHY | HTRT | U | F | 0.028 d |
| Golden Hamster |  |  |  |  |  |  |  |  | 2 ug/kg/mi |  |
| Ovis aries | NONE | LAB | PU | 2 | NOEL | PHY | GPHY | U | F | 0.01 d |
| Domestic Sheep |  |  |  |  |  |  |  |  | 2 mg/ml |  |
| Canis familiaris | NONE | LAB | OP | 2 | NOEL | PHY | KEXC | U | F | 0.01 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.5 ug/kg/mi |  |
| Canis familiaris | NONE | LAB | OP | 2 | NOEL | PHY | KEXC | U | F | 0.01 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.5 ug/kg/mi |  |
| Canis familiaris | NONE | LAB | OP | 2 | NOEL | PHY | NAEX | U | F | 0.01 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.5 ug/kg/mi |  |
| Canis familiaris | NONE | LAB | OP | 2 | NOEL | PHY | NAEX | U | F | 0.01 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.5 ug/kg/mi |  |
| Canis familiaris | NONE | LAB | OP | 2 | NOEL | PHY | KEXC | U | F | 0.01 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.5 ug/kg/mi |  |
| Canis familiaris | NONE | LAB | OP | 2 | NOEL | PHY | KEXC | U | F | 0.01 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.5 ug/kg/mi |  |
| Canis familiaris | NONE | LAB | OP | 2 | NOEL | PHY | BLPR | U | F | 0.01 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.5 ug/kg/mi |  |
| Canis familiaris | NONE | LAB | OP | 2 | NOEL | PHY | BDFW | U | F | 0.01 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.5 ug/kg/mi |  |
| Canis familiaris | NONE | LAB | OP | 2 | NOEL | PHY | CLEX | U | F | 0.01 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.5 ug/kg/mi |  |
| Canis familiaris | NONE | LAB | OP | 2 | NOEL | PHY | CLEX | U | F | 0.01 d |
| Domestic Dog |  |  |  |  |  |  |  |  | 0.5 ug/kg/mi |  |
| Mus musculus | NONE | LAB | IP | 2 | NOEL | REP | PROG | U | F | 0.014 d |
| House Mouse |  |  |  |  |  |  |  |  | 1.3 mg/kg |  |
| **Mammals; Standard Test Species** | | | | | | | | | | |
| Rattus norvegicus | NONE | LAB | GV | 9 | LD50 | MOR | MORT | U | F | 1 d |
| Norway Rat |  |  |  |  |  |  |  |  | 19.3 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IJ | 2 | LOEL | BCM | DOPC | U | F | 0.007 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.8 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | LOEL | BCM | TAUR | U | F | 1 brd |
| Norway Rat |  |  |  |  |  |  |  |  | 0.5 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IP | 5 | LOEL | BCM | TAUR | U | F | 0.014 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.30 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | LOEL | BCM | DOPC | U | F | 0.167 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.5 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IV | 2 | LOEL | BCM | GLUC | U | F | 0.004 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.05 mg/org |  |
| Rattus norvegicus | NONE | LAB | SC | 3 | LOEL | BEH | LOCO | U | F | 0.021 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.15 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 3 | LOEL | BEH | LOCO | U | F | 0.021 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.15 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 3 | LOEL | BEH | LOCO | U | F | 0.021 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.15 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 3 | LOEL | BEH | LOCO | U | F | 0.021 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.15 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | LOEL | BEH | HMVT | U | F | 0.021 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IP | 2 | LOEL | BEH | ACTV | U | F | 0.01 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.20 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IP | 2 | LOEL | BEH | ACTV | U | F | 0.01 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.20 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 3 | LOEL | BEH | LOCO | U | F | 0.042 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.5 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | LOEL | BEH | ACTV | U | F | 0.001 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 3 | LOEL | BEH | NMVM | U | F | 0.031 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.4 mg/kg bdwt |  |
| Rattus norvegicus | NONE | LAB | SC | 3 | LOEL | BEH | NMVM | U | F | 0.031 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.2 mg/kg bdwt |  |
| Rattus norvegicus | NONE | LAB | IP | 6 | LOEL | BEH | ACTP | U | F | 0.01 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.5 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | LOEL | BEH | HMVT | U | F | 0.097 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.5 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | LOEL | CEL | RSBC | U | F | 0.125 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | LOEL | CEL | RSBC | U | F | 1 brd |
| Norway Rat |  |  |  |  |  |  |  |  | 0.5 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | LOEL | ENZ | EROD | U | A | 0.5 d |
| Norway Rat |  |  |  |  |  |  |  |  | 2.5 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | LOEL | ENZ | EROD | U | A | 0.5 d |
| Norway Rat |  |  |  |  |  |  |  |  | 2.5 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | LOEL | ENZ | EROD | U | A | 0.5 d |
| Norway Rat |  |  |  |  |  |  |  |  | 2.5 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 8 | LOEL | ENZ | ORDC | U | F | 0.167 d |
| Norway Rat |  |  |  |  |  |  |  |  | 3.3 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | LOEL | ENZ | ORDC | U | F | 0.125 d |
| Norway Rat |  |  |  |  |  |  |  |  | 3 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | LOEL | ENZ | TSHX | U | F | 0.01 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IP | 2 | LOEL | GEN | MRNA | U | F | 0.5 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.60 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IP | 2 | LOEL | GEN | DNAB | U | F | 0.042 d |
| Norway Rat |  |  |  |  |  |  |  |  | 3 mg/kg/d |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | LOEL | GRO | GAIN | U | F | 1 d |
| Norway Rat |  |  |  |  |  |  |  |  | 3 mg/kg |  |
| Rattus norvegicus | NONE | LAB | DM | 2 | LOEL | GRO | WGHT | U | F | 1 d |
| Norway Rat |  |  |  |  |  |  |  |  | 5 mg/kg/d |  |
| Rattus norvegicus | NONE | LAB | IP | 2 | LOEL | HRM | ESTR | U | F | 0.792 d |
| Norway Rat |  |  |  |  |  |  |  |  | 6.25 ng/g bdwt |  |
| Rattus norvegicus | NONE | LAB | IP | 2 | LOEL | HRM | ESTR | U | F | 0.792 d |
| Norway Rat |  |  |  |  |  |  |  |  | 6.25 ng/g bdwt |  |
| Rattus norvegicus | NONE | LAB | IV | 2 | LOEL | HRM | EPIN | U | F | 0.001 d |
| Norway Rat |  |  |  |  |  |  |  |  | 100 ug/kg |  |
| Rattus norvegicus | NONE | LAB | IV | 2 | LOEL | HRM | GHRM | U | F | 0.01 d |
| Norway Rat |  |  |  |  |  |  |  |  | 20 ug/mi |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | LOEL | HRM | CORT | U | F | 0.01 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | LOEL | HRM | CORT | U | F | 0.01 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IV | 2 | LOEL | HRM | EPIN | U | F | 0.001 d |
| Norway Rat |  |  |  |  |  |  |  |  | 30 nmol |  |
| Rattus norvegicus | NONE | LAB | IV | 5 | LOEL | HRM | EPIN | U | F | 0.001 d |
| Norway Rat |  |  |  |  |  |  |  |  | 30 nmol |  |
| Rattus norvegicus | NONE | LAB | IA | 2 | LOEL | HRM | EPIN | U | F | 1 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IA | 2 | LOEL | HRM | EPIN | U | F | 1 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IA | 3 | LOEL | HRM | EPIN | U | F | 0.001 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 4 | LOEL | HRM | CORT | U | F | 0.021 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | LOEL | HRM | CORT | U | F | 0.021 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | LOEL | HRM | NORE | U | F | 0.042 d |
| Norway Rat |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 3 | LOEL | HRM | NORE | U | F | 0.042 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IP | 2 | LOEL | HRM | CORT | U | F | 0.014 d |
| Norway Rat |  |  |  |  |  |  |  |  | 5 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IP | 2 | LOEL | HRM | CORT | U | F | 0.004 d |
| Norway Rat |  |  |  |  |  |  |  |  | 5 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IJ | 3 | LOEL | HRM | CORT | U | F | 0.014 d |
| Norway Rat |  |  |  |  |  |  |  |  | 250 nmol/org |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | LOEL | HRM | DOPA | U | F | 0.167 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.5 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 4 | LOEL | IMM | GIMM | U | F | 0.021 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | LOEL | IMM | GIMM | U | F | 0.021 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IV | 2 | LOEL | MPH | DMTR | U | F | 0.001 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.05 mg/org |  |
| Rattus norvegicus | NONE | LAB | IP | 2 | LOEL | PHY | BTMP | U | F | 0.083 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.25 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IV | 4 | LOEL | PHY | GPHY | U | F | 0.031 d |
| Norway Rat |  |  |  |  |  |  |  |  | 10 ug/kg |  |
| Rattus norvegicus | NONE | LAB | IV | 5 | LOEL | PHY | VSCR | U | F | 0.031 d |
| Norway Rat |  |  |  |  |  |  |  |  | 10 ug/kg |  |
| Rattus norvegicus | NONE | LAB | IV | 4 | LOEL | PHY | GPHY | U | F | 0.031 d |
| Norway Rat |  |  |  |  |  |  |  |  | 30 ug/kg |  |
| Rattus norvegicus | NONE | LAB | IV | 4 | LOEL | PHY | BLPR | U | F | 0.031 d |
| Norway Rat |  |  |  |  |  |  |  |  | 30 ug/kg |  |
| Rattus norvegicus | NONE | LAB | IV | 3 | LOEL | PHY | CDIN | U | F | 0.031 d |
| Norway Rat |  |  |  |  |  |  |  |  | 300 ug/kg |  |
| Rattus norvegicus | NONE | LAB | IV | 5 | LOEL | PHY | BLPR | U | F | 0.031 d |
| Norway Rat |  |  |  |  |  |  |  |  | 100 ug/kg |  |
| Rattus norvegicus | NONE | LAB | IV | 2 | LOEL | PHY | BLPR | U | F | 0.007 d |
| Norway Rat |  |  |  |  |  |  |  |  | 20 ug/mi |  |
| Rattus norvegicus | NONE | LAB | SC | 3 | LOEL | PHY | BDVL | U | A | 0.014 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.5 mg/kg bdwt |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | LOEL | PHY | OXYT | U | A | 0.031 d |
| Norway Rat |  |  |  |  |  |  |  |  | 5.0 mg/kg bdwt |  |
| Rattus norvegicus | NONE | LAB | IV | 3 | LOEL | PHY | HTRT | U | F | 0.001 d |
| Norway Rat |  |  |  |  |  |  |  |  | 120 nmol |  |
| Rattus norvegicus | NONE | LAB | IA | 3 | LOEL | PHY | HTRT | U | F | 0.001 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 3 | LOEL | PHY | BLPR | U | A | 0.063 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.5 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IP | 2 | LOEL | PHY | VENT | U | F | 0.004 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.60 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IV | 3 | LOEL | PHY | DGST | U | F | 0.001 d |
| Norway Rat |  |  |  |  |  |  |  |  | 1 nmol/org |  |
| Rattus norvegicus | NONE | LAB | IV | 3 | LOEL | PHY | DGST | U | F | 0.001 d |
| Norway Rat |  |  |  |  |  |  |  |  | 1 nmol/org |  |
| Rattus norvegicus | NONE | LAB | IV | 5 | LOEL | PHY | DGST | U | F | 0.008 d |
| Norway Rat |  |  |  |  |  |  |  |  | 300 nmol/kg |  |
| Rattus norvegicus | NONE | LAB | IV | 2 | LOEL | PHY | GPHY | U | F | 0.125 d |
| Norway Rat |  |  |  |  |  |  |  |  | <= 10 ug/kg/mi |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | LOEL | PHY | PRSY | U | F | 1 brd |
| Norway Rat |  |  |  |  |  |  |  |  | 0.5 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IJ | 7 | LOEL | PHY | HTRT | U | F | 0.042 d |
| Norway Rat |  |  |  |  |  |  |  |  | 3.696 pmol |  |
| Rattus norvegicus | NONE | LAB | IJ | 7 | LOEL | PHY | HTRT | U | F | 0.042 d |
| Norway Rat |  |  |  |  |  |  |  |  | 12.3 pmol |  |
| Rattus norvegicus | NONE | LAB | IJ | 7 | LOEL | PHY | HTRT | U | F | 0.042 d |
| Norway Rat |  |  |  |  |  |  |  |  | 12.3 pmol |  |
| Rattus norvegicus | NONE | LAB | IJ | 7 | LOEL | PHY | HTRT | U | F | 0.042 d |
| Norway Rat |  |  |  |  |  |  |  |  | 12.3 pmol |  |
| Rattus norvegicus | NONE | LAB | IJ | 7 | LOEL | PHY | HTRT | U | F | 0.042 d |
| Norway Rat |  |  |  |  |  |  |  |  | 369 pmol |  |
| Rattus norvegicus | NONE | LAB | IV | 2 | LOEL | PHY | EXCR | U | F | 0.021 d |
| Norway Rat |  |  |  |  |  |  |  |  | 2 ug/kg/mi |  |
| Rattus norvegicus | NONE | LAB | IV | 2 | LOEL | PHY | NRXN | U | NC | 0.021 d |
| Norway Rat |  |  |  |  |  |  |  |  | 2 ug/kg/mi |  |
| Rattus norvegicus | NONE | LAB | IV | 6 | LOEL | PHY | BLPR | U | F | 0.021 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.0833 ug/kg |  |
| Rattus norvegicus | NONE | LAB | IV | 2 | LOEL | PHY | BLPR | U | F | 0.001 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.05 mg/org |  |
| Rattus norvegicus | NONE | LAB | IP | 2 | LOEL | REP | OVRT | U | F | 0.792 d |
| Norway Rat |  |  |  |  |  |  |  |  | 6.25 ng/g bdwt |  |
| Rattus norvegicus | NONE | LAB | IP | 2 | LOEL | REP | OVRT | U | F | 0.792 d |
| Norway Rat |  |  |  |  |  |  |  |  | 6.25 ng/g bdwt |  |
| Rattus norvegicus |  | LAB | DT | 2 | LOEL | REP | PSTR | U | F | 1.57 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.2 mg/100g bw/d |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | NOEC | CEL | RSBC | U | F | 0.01 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | NOEC | ENZ | TSHX | U | F | 0.01 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | NOEC | ENZ | TSHX | U | F | 0.01 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | NOEC | HRM | DOPA | U | F | 0.01 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | NOEC | HRM | DOPA | U | F | 0.01 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | NOEL | BCM | PRCO | U | F | 0.125 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IP | 5 | NOEL | BCM | TAUR | U | F | 0.014 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.15 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 5 | NOEL | BEH | GBHV | U | F | 0.031 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.5 mg/kg bdwt |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | NOEL | BEH | GBHV | U | F | 0.004 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.375 mg/kg bdwt |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | NOEL | BEH | HMVT | U | F | 0.021 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 3 | NOEL | BEH | LOCO | U | F | 0.01 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.8 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 3 | NOEL | BEH | NMVM | U | F | 0.031 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.4 mg/kg bdwt |  |
| Rattus norvegicus | NONE | LAB | SC | 3 | NOEL | BEH | NMVM | U | F | 0.031 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.4 mg/kg bdwt |  |
| Rattus norvegicus | NONE | LAB | SC | 3 | NOEL | BEH | NMVM | U | F | 0.031 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.2 mg/kg bdwt |  |
| Rattus norvegicus | NONE | LAB | IP | 6 | NOEL | BEH | ACTP | U | F | 0.01 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.25 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | NOEL | CEL | RSBC | U | F | 0.01 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 8 | NOEL | ENZ | ORDC | U | F | 0.167 d |
| Norway Rat |  |  |  |  |  |  |  |  | ~ 1.5 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | NOEL | ENZ | ORDC | U | F | 0.25 d |
| Norway Rat |  |  |  |  |  |  |  |  | 3 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | NOEL | FDB | FCNS | U | F | 0.125 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | NOEL | GRO | WGHT | U | F | 0.125 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IP | 2 | NOEL | HRM | ESTR | U | F | 0.792 d |
| Norway Rat |  |  |  |  |  |  |  |  | 6.25 ng/g bdwt |  |
| Rattus norvegicus | NONE | LAB | IA | 2 | NOEL | HRM | EPIN | U | F | 1 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 4 | NOEL | HRM | CORT | U | F | 0.021 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.2 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IJ | 2 | NOEL | HRM | DOPA | U | F | 0.007 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.8 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | NOEL | HRM | DOPA | U | F | 0.01 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 4 | NOEL | IMM | GIMM | U | F | 0.021 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.2 mg/kg |  |
| Rattus norvegicus | NONE | LAB | SC | 2 | NOEL | MPH | WGHT | U | F | 0.125 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IV | 3 | NOEL | PHY | ATFL | U | F | 0.042 d |
| Norway Rat |  |  |  |  |  |  |  |  | 40 ug/kg/mi |  |
| Rattus norvegicus | NONE | LAB | IV | 4 | NOEL | PHY | GPHY | U | F | 0.031 d |
| Norway Rat |  |  |  |  |  |  |  |  | 10 ug/kg |  |
| Rattus norvegicus | NONE | LAB | IV | 4 | NOEL | PHY | BLPR | U | F | 0.031 d |
| Norway Rat |  |  |  |  |  |  |  |  | 10 ug/kg |  |
| Rattus norvegicus | NONE | LAB | IV | 3 | NOEL | PHY | CDIN | U | F | 0.031 d |
| Norway Rat |  |  |  |  |  |  |  |  | 100 ug/kg |  |
| Rattus norvegicus | NONE | LAB | IV | 5 | NOEL | PHY | BLPR | U | F | 0.031 d |
| Norway Rat |  |  |  |  |  |  |  |  | 30 ug/kg |  |
| Rattus norvegicus | NONE | LAB | GV | 3 | NOEL | PHY | TRAN | U | F | 1 d |
| Norway Rat |  |  |  |  |  |  |  |  | 7.5 mg/kg |  |
| Rattus norvegicus | NONE | LAB | IJ | 7 | NOEL | PHY | HTRT | U | F | 0.042 d |
| Norway Rat |  |  |  |  |  |  |  |  | 3.696 pmol |  |
| Rattus norvegicus | NONE | LAB | IJ | 7 | NOEL | PHY | HTRT | U | F | 0.042 d |
| Norway Rat |  |  |  |  |  |  |  |  | 3.696 pmol |  |
| Rattus norvegicus | NONE | LAB | IJ | 7 | NOEL | PHY | HTRT | U | F | 0.042 d |
| Norway Rat |  |  |  |  |  |  |  |  | 3.696 pmol |  |
| Rattus norvegicus | NONE | LAB | IJ | 7 | NOEL | PHY | HTRT | U | F | 0.042 d |
| Norway Rat |  |  |  |  |  |  |  |  | 123 pmol |  |
| Rattus norvegicus | NONE | LAB | IV | 6 | NOEL | PHY | BLPR | U | F | 0.021 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.0167 ug/kg |  |
| Rattus norvegicus | NONE | LAB | IV | 5 | NOEL | PHY | BLPR | U | F | 0.006 d |
| Norway Rat |  |  |  |  |  |  |  |  | 20 umol/kg |  |
| Rattus norvegicus | NONE | LAB | IP | 2 | NOEL | REP | OVRT | U | F | 0.792 d |
| Norway Rat |  |  |  |  |  |  |  |  | 6.25 ng/g bdwt |  |
| Rattus norvegicus | NONE | LAB | SC | 2 |  | ACC | RSDE | U | F | 0.01 d |
| Norway Rat |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | NONE | LAB | GV | 9 | NR-LETH | MOR | MORT | U | F | 1 d |
| Norway Rat |  |  |  |  |  |  |  |  | 60.0 mg/kg |  |

As taken from the US EPA ECOTOX database

## *5.2.* *Repeated dose toxicity*

“Smoking is a preventable risk factor for stroke and smoking-derived nicotine exacerbates post-ischemic damage via inhibition of estrogen receptor beta (ER-&beta;) signaling in the brain of female rats. ER-&beta; regulates inflammasome activation in the brain. Therefore, we hypothesized that chronic nicotine exposure activates the inflammasome in the brain, thus exacerbating ischemic brain damage in female rats. To test this hypothesis, adult female Sprague-Dawley rats (6⁻7 months old) were exposed to nicotine (4.5 mg/kg/day) or saline for 16 days. Subsequently, brain tissue was collected for immunoblot analysis. In addition, another set of rats underwent transient middle cerebral artery occlusion (tMCAO; 90 min) with or without nicotine exposure. One month after tMCAO, histopathological analysis revealed a significant increase in infarct volume in the nicotine-treated group (64.24 &plusmn; 7.3 mm³; mean &plusmn; SEM; n = 6) compared to the saline-treated group (37.12 &plusmn; 7.37 mm³; n = 7, p < 0.05). Immunoblot analysis indicated that nicotine increased cortical protein levels of caspase-1, apoptosis-associated speck-like protein containing a CARD (ASC) and pro-inflammatory cytokines interleukin (IL)-1&beta; by 88% (p < 0.05), 48% (p < 0.05) and 149% (p < 0.05), respectively, when compared to the saline-treated group. Next, using an in vitro model of ischemia in organotypic slice cultures, we tested the hypothesis that inhibition of nicotine-induced inflammasome activation improves post-ischemic neuronal survival. Accordingly, slices were exposed to nicotine (100 ng/mL; 14⁻16 days) or saline, followed by treatment with the inflammasome inhibitor isoliquiritigenin (ILG; 24 h) prior to oxygen-glucose deprivation (OGD; 45 min). Quantification of neuronal death demonstrated that inflammasome inhibition significantly decreased nicotine-induced ischemic neuronal death. Overall, this study shows that chronic nicotine exposure exacerbates ischemic brain damage via activation of the inflammasome in the brain of female rats.” (d’Adesky et al. 2018).

“Parkinson's disease (PD) is characterized by the degeneration of the dopaminergic neurons in the substantia nigra pars compacta (SNpc). Clinical and experimental evidence suggest that the activation of the nicotinic acetylcholine receptor (nAChR) could be protective for PD. In this study, we investigated the neuroprotective capacity of nicotine in a rat PD model. Considering that iron metabolism has been implicated in PD pathophysiology and nicotine has been described to chelate this metal, we also studied the effect of nicotine on the cellular labile iron pool (LIP) levels. Rotenone (1 μg) was unilaterally injected into the median forebrain bundle to induce the degeneration of the nigrostriatal pathway. Nicotine administration (1 mg/K, s.c. daily injection, starting 5 days before rotenone and continuing for 30 days) attenuated the dopaminergic cell loss in the SNpc and the degeneration of the dopaminergic terminals provoked by rotenone, as assessed by immunohistochemistry. Furthermore, nicotine partially prevented the reduction on dopamine levels in the striatum and improved the motor deficits, as determined by HPLC-ED and the forelimb use asymmetry test, respectively. Studies in primary mesencephalic cultures showed that pretreatment with nicotine (50 μM) improved the survival of tyrosine hydroxylase-positive neurons after rotenone (20 nM) exposure. Besides, nicotine induced a reduction in the LIP levels assessed by the calcein dequenching method only at the neuroprotective dose. These effects were prevented by addition of the nAChRs antagonist mecamylamine (100 μM). Overall, we demonstrate a neuroprotective effect of nicotine in a model of PD in rats and that a reduction in iron availability could be an underlying mechanism.” (Mouhape et al. 2019).

“Long-term exposure to cigarette smoke induces severe injuries to respiratory system through several mechanisms, some of them are well defined, but many others are not yet elucidated. Beside its classical role in nervous system, we have previously shown that Nerve Growth Factor (NGF) and its receptors have a crucial role in airway inflammatory diseases, such as Chronic Obstructive Pulmonary Disease. To expand our knowledge about the relevance of NGF and its receptors in airway diseases induced by cigarette smoking, we exposed for 16 weeks the bronchial epithelial cell line BEAS-2B to sub-toxic concentrations of whole cigarette smoke extract or pure nicotine. Viability, cell cycle gene expression, cell morphology and migration ability were tested and compared to NGF release and gene expression. Modulation of its receptors TrKA and p75NTR was also analyzed. The present study shows that long term exposure of BEAS-2B cells to cigarette smoke extract or nicotine induces: (A) differences: in cell viability, in the expression of cell cycle-related genes, in NGF release and in gene expression of NGF and its receptors; (B) similarities: in morphology and migration ability. Taken together, our data provide new insights about the biological role of NGF and its receptors in airway diseases induced by long-term cigarette smoking and, finally, our data evidence the opportunity not to use nicotine lozenges or e-cigarettes as anti smoking replacement therapy in patients with a previous airway disease according to the ability of nicotine to increase the amount of the pro-inflammatory cytokine NGF into the bronchial environment.” (Stabile et al. 2018).

“CONTEXT: Cigarette smoking is considered to be a major risk factor for the development of diabetes and cardiovascular disease. Oestrogen-progestin combined oral contraceptive (COC) use has been associated with adverse cardiometabolic events. OBJECTIVE: We hypothesized that nicotine would ameliorate insulin resistance (IR) that is accompanied by decreased cardiac glycogen synthase kinase-3 (GSK-3) and plasminogen activator inhibitor-1 (PAI-1). METHODS: Female Wistar rats received (po) low-(0.1 mg/kg) or high-nicotine (1.0 mg/kg) with or without COC containing 5.0 µg levonorgestrel plus 1.0 µg ethinylestradiol daily for 8 weeks. RESULTS: Data showed that COC treatment or nicotine exposure led to IR, glucose deregulation, atherogenic dyslipidemia, increased corticosterone, aldosterone, cardiac and circulating GSK-3 values and PAI-1. However, these effects with the exception of corticosterone and aldosterone were ameliorated in COC + nicotine-exposed rats. CONCLUSION: Amelioration of IR induced by COC treatment is accompanied by decreased circulating PAI-1, cardiac PAI-1 and GSK-3 instead of circulating aldosterone and corticosterone.” (Michael and Olatunji 2018).

“E-cigarette usage is increasing, especially among the young, with both the general population and physicians perceiving them as a safe alternative to tobacco smoking. Worryingly, e-cigarettes are commonly used by pregnant women. As nicotine is known to adversely affect children in utero, we hypothesized that nicotine delivered via e-cigarettes would negatively affect lung development. To test this, we developed a mouse model of maternal e-vapor (nicotine and nicotine-free) exposure and investigated the impact on the growth and lung inflammation in both offspring and mothers. Female Balb/c mice were exposed to e-fluid vapor containing nicotine (18 mg/ml nicotine E-cigarette [E-cig18], equivalent to two cigarettes per treatment, twice daily,) or nicotine free (E-cig0 mg/ml) from 6 weeks before mating until pups weaned. Male offspring were studied at Postnatal Day (P) 1, P20, and at 13 weeks. The mothers were studied when the pups weaned. In the mothers' lungs, e-cigarette exposure with and without nicotine increased the proinflammatory cytokines IL-1β, IL-6, and TNF-α. In adult offspring, TNF-α protein levels were increased in both E-cig18 and E-cig0 groups, whereas IL-1β was suppressed. This was accompanied by global changes in DNA methylation. In this study, we found that e-cigarette exposure during pregnancy adversely affected maternal and offspring lung health. As this occurred with both nicotine-free and nicotine-containing e-vapor, the effects are likely due to by-products of vaporization rather than nicotine.” (Chen H et al. 2018).

“Estrogen-progestin oral contraceptives (COC) or tobacco smoking has been associated with hypertension and endothelial dysfunction resulting in increased risk of cardiovascular diseases (CVD). Contrasting effects of nicotine exposure on endothelial function have been reported. The effect of non-smoking nicotine exposure on endothelial dysfunction during COC treatment remains to be fully elucidated. We therefore, sought to determine the effects of nicotine exposure during COC treatment on endothelial dysfunction mediators and circulating corticosteroids. Female Wistar rats aged 10 weeks were given (po) vehicle, nicotine (1.0 mg/kg) with or without COC steroids (1.0 µg ethinylestradiol and 5.0 µg levonorgestrel) daily for 6 weeks. Nicotine exposure caused 113.3% increase in insulinemia whereas COC treatment led to 76.9% increased insulinemia compared with control. Furthermore, COC treatment or nicotine exposure led to glucose deregulation, insulin resistance, reduced nitric oxide bioavailability, elevated plasminogen activator inhibitor-1, uric acid, oxidative stress, atherogenic dyslipidemia, and corticosteroids. However, COC + NIC treatment led to 41.2% decrease in insulemina compared with COC-treated rats. Furthermore, all other alterations were alleviated by nicotine exposure in COC-treated female rats with the exception of corticosteroids.” (Michael and Olatunji 2018).

“Use of tobacco products is injurious to health in men and women. However, tobacco use by pregnant women receives greater scrutiny because it can also compromise the health of future generations. More men smoke cigarettes than women. Yet the impact of nicotine use by men upon their descendants has not been as widely scrutinized. We exposed male C57BL/6 mice to nicotine (200 μg/mL in drinking water) for 12 wk and bred the mice with drug-naïve females to produce the F1 generation. Male and female F1 mice were bred with drug-naïve partners to produce the F2 generation. We analyzed spontaneous locomotor activity, working memory, attention, and reversal learning in male and female F1 and F2 mice. Both male and female F1 mice derived from the nicotine-exposed males showed significant increases in spontaneous locomotor activity and significant deficits in reversal learning. The male F1 mice also showed significant deficits in attention, brain monoamine content, and dopamine receptor mRNA expression. Examination of the F2 generation showed that male F2 mice derived from paternally nicotine-exposed female F1 mice had significant deficits in reversal learning. Analysis of epigenetic changes in the spermatozoa of the nicotine-exposed male founders (F0) showed significant changes in global DNA methylation and DNA methylation at promoter regions of the dopamine D2 receptor gene. Our findings show that nicotine exposure of male mice produces behavioral changes in multiple generations of descendants. Nicotine-induced changes in spermatozoal DNA methylation are a plausible mechanism for the transgenerational transmission of the phenotypes. These findings underscore the need to enlarge the current focus of research and public policy targeting nicotine exposure of pregnant mothers by a more equitable focus on nicotine exposure of the mother and the father.” (McCarthy et al. 2018).

“The aim of this study was to evaluate the effect of acute administration of nicotine and ethanol on tooth movement in rats. Two hundred rats were divided into eight groups: S: saline; N: nicotine; E: ethanol; NE: nicotine and ethanol; SM: saline with tooth movement; NM: nicotine with tooth movement; EM: ethanol with tooth movement; and NEM: nicotine and ethanol with tooth movement. All the solutions were applied for 32, 44, or 58 days, according to the subgroup. Orthodontic movement (25 cN) was initiated 30 days after solution administration in the groups with tooth movement. The rats were euthanized 2, 14, or 28 days after initiation of tooth movement. Tooth sections were stained using picrosirius and tartrate-resistant acid phosphatase (TRAP). The data were compared by ANOVA using Tukey's HSD and Games-Howell. On day 28 of tooth movement, the NEM group had a lower percentage of type I collagen compared to the SM group (p = 0.0448), and the S group had a higher number of osteoclasts/μm2 compared to the N group (p = 0.0405). Nicotine and ethanol did not affect the tooth movement rate, regardless of induction of orthodontic movement. Nicotine influenced the number of osteoclasts by decreasing their quantity when dental movement was not induced. When nicotine was associated with ethanol, it interfered in the maturation of collagen fibers during orthodontic movement.” (Araujo et al. 2018).

“Prenatal nicotine exposure (PNE) can cause hypersensitivity of hypothalamic-pituitary-adrenal (HPA) axis in offspring with intrauterine growth retardation. The purpose of this study was to explore the original mechanism of intrauterine development that mediates hypersensitivity of the HPA axis in offspring due to PNE. Pregnant Wistar rats were injected subcutaneously with 2 mg/kg·d of nicotine on the 9th to the 20th gestational day (GD9-GD20) and the fetuses were extracted at GD20. Compared with the control group, fetal rats by PNE showed increased hippocampal apoptosis, reduced synaptic plasticity and downregulation of the brain-derived neurotrophic factor (BDNF) pathway, whereas glutamic acid decarboxylase 67 (GAD67) expression was upregulated. Rat fetal hippocampal H19-7/IGF1R cell lines were treated with different concentrations of nicotine (1, 10 and 100 μM) for 3 days, the extracellular fluid glutamate (Glu) level increased and similar effects were observed as in vivo. Intervention treatments caused the opposite results. These results indicated that PNE downregulates the BDNF pathway and mediates the hippocampal excitotoxicity; then, the compensatory upregulation of GAD67 causes the imbalance of signal output in the fetal hippocampus. The negative feedback regulation of the paraventricular hypothalamic nucleus by the hippocampus is unbalanced, eventually causing hypersensitivity of the HPA axis of the offspring.” (Pei et al. 2019).

“Candida albicans ATCC 14053 and Candida parapsilosis ATCC 22019 hyphal-wall protein 1 (HWP1) are involved in hyphae formation and pathogenesis. The transcriptional agglutinin-like sequence 3 (ALS3) genes in both species are responsible for the development of biofilm and colonization on tooth surfaces. Therefore, we investigated the expression of HWP1 and ALS3 quantitatively in C. albicans and C. parapsilosis and examined the biofilm structure upon exposure to various nicotine concentrations. In vitro, biofilms of Candida species were developed directly on slides using the Lab-Tek Chamber Slide System and visualized by confocal laser scanning microscopy. Quantitative real-time polymerase chain reaction was used to measure HWP1 and ALS3 expression in C. albicans ATCC 14053 and C. parapsilosis ATCC 22019. The results indicated that nicotine multiplied the number of yeast cells and increased the extracellular polysaccharides of Candida species. We also found that 1-2 mg/mL nicotine could enhance the formation of biofilm. The findings also revealed that the expression of HWP1 and ALS3 in Candida species were increased as the nicotine concentration increased. Therefore, nicotine influences the biofilm development of oral-associated C. albicans ATCC 14053 and C. parapsilosis ATCC 22019.” (Gunasegar et al. 2019).

“INTRODUCTION: Nicotine stimulates fibroblast proliferation while increasing inflammation and fibrosis of tissues. The cannabinoid receptor 1 (CB1R) is mainly located in the CNS, while cannabinoid receptor 2 (CB2R) is located in the immune cells within the body. CB2R regulates inflammatory processes and fibroblast function. PURPOSE: We investigated the impact of CB2R agonist, JWH 133 and the antagonist, AM630 on lung tissue, applied directly before nicotine application. MATERIAL AND METHODS: 40 mice were placed into 4 groups. The experimental groups received nicotine intraperitoneally at a dose of 0.05 mg/kg of body weight (BW) for 14 days. Group B also received AM630 (0.5mg/kg of BW), while Group A was administered with JWH133 (1 mg/kg of BW). Group N received nicotine alone. The Control group C received 0.9% NaCl. After decapitation, lung tissues were stained with H&E, Trichrome Masson's method, and IHC against CTGF and α-SMA. The digital image processing system Image J with the IHC profiler plugins was then employed, optical density and IHC optical density score were calculated. RESULTS: In the N group, an increase in the thickness of alveolar spaces (9.16 SD4.95µm vs. 4.77SD2.99µm in the C group), leukocytes infiltration and collagen deposition has been observed (OD: 0.20 SD0.0vs 0.07SD0.04 in the C group). In the B group, the alveolar space thickness has been the highest (11.57SD8.13µm). Furthermore, in this group, hyperaemia, destruction of lung structure, hyperplasia of II type pneumocyte and interstitial fibrosis has been observed (OD: 0.23 SD0.08). In contrast, the lung tissue of the A group has had normal structure and the thinnest alveolar septum (3.88 SD2.64µm). The expression of CTGF and α-SMA has been the highest in the B group. CONCLUSION: Nicotine induces interstitial lung fibrosis that is enhanced by the CB2R antagonist and diminished by the CB2R agonist. Therefore, the CB2R agonist may offer a protection against fibrosis.” (Wawryk-Gawda et al. 2018).

“INTRODUCTION: Nicotine is an alkaloid that affects the functioning of the central nervous system and produces dependence. In low doses, it acts as a stimulant and relaxant. Nicotine was reported to have pro-cognitive effects in humans and animals. However, high doses of nicotine are harmful for many organs. The aim of the study was to check whether a 30-day exposure to transdermal nicotine affects memory and biochemical parameters in mice. MATERIAL AND METHODS: A total of 32 mice (16 males and 16 females) were used in the experiment. Mice were divided into 4 groups of 8 animals each: I control-females receiving placebo patches for 30 days, II females receiving nicotine patches for 30 days, III control-males receiving placebo patches, IV males receiving nicotine patches. Spontaneous alternation and locomotor activity were examined weekly in a Y-maze. Body mass was recorded daily. On day 30, venous blood samples were obtained and the animals were anaesthetized with CO<sub>2</sub>. Their blood was used to measure alanine transaminase (ALT), asparagine transaminase (AST), cholesterol, creatinine and glycosylated haemoglobin (HbA<sub>1</sub>C). RESULTS: Nicotine significantly improved memory in male mice on day 8. It increased ALT and AST activities in males and females, as well as the concentration of cholesterol in their blood sera. CONCLUSIONS: In conclusion, transdermal nicotine may produce transient improvement in fresh spatial memory in male mice, but it is not a long-term effect and therefore nicotine does not seem to be appropriate for use in the treatment of neurodegenerative disorders. It elevates blood cholesterol level and thus may increase the risk of atherosclerosis and cardiovascular events; moreover, it negatively affects liver enzymes. Nicotine use is therefore not recommended.” (Nieradko-Iwanicka et al. 2019).

“Prenatal nicotine exposure (PNE) is closely related to depression in offspring. However, the underlying mechanism is still unclear. We hypothesized that neurosteroid in the hippocampus may mediate PNE‑induced depression‑like behaviors. Nicotine was subcutaneously administered (1.0 mg/kg) to pregnant rats twice daily from gestational day (GD) 9 to 20. In adolescent offspring, PNE significantly increased immobility time and decreased the sucrose preference in female rats. The numbers of hippocampal neurons declined in the CA3 and DG regions. Steroidogenic acute regulatory protein (StAR) expression was suppressed in female rats. In fetal offspring, the neuronal numbers of CA3 regions in PNE female fetal hippocampal were significantly decreased, accompanied by the enhanced content of corticosterone and StAR expression. These data indicated that PNE induced depression‑like behavior in adolescent female rats via the regulation of neurosteroid levels in the hippocampus.” (Zhang C et al. 2019).

“Prenatal nicotine exposure (PNE) could induce ovarian dysplasia in offspring. This study aimed to confirm its intrauterine origin and explore a programming mechanism of ovarian dysplasia caused by PNE. Pregnant Wistar rats were injected subcutaneously with nicotine (2 mg/kg.d) from gestation day (GD) 9 to GD20. Serum of female offspring was obtained for hormone assays and ovarian tissues were collected. The results showed that PNE impaired ovarian development, and inhibited estradiol production and cytochrome P450 aromatase (P450arom) expression before and after birth. Moreover, the nicotinic acetylcholine receptors (nAChRs) expression was increased in utero, while histone 3 lysine 9 acetylation (H3K9ac) and H3K27ac levels in the P450arom promoter region were decreased persistently in PNE group before and after birth. In vitro, nicotine decreased P450arom expression and estradiol production in human granulosa cell line KGN. Furthermore, nicotine treatment up-regulated nAChRα6 and α9 expression and down-regulated the H3K9ac and H3K27ac levels of the P450arom promoter region. Non-specific nAChRs inhibitor vecuronium bromide reversed these effects. These results suggest that PNE could induce ovarian dysplasia and inhibit estradiol synthesis in the female offspring rats, which was related to the decreased H3K9ac and H3K27ac levels in the promotor region of the P450arom via the nAChRs.” (Fan et al. 2019).

“BACKGROUND: Nicotine is associated with increased incidence of periodontal disease and poor response to therapy. This article aimed at identifying the expression of matrix metalloproteinases 2 (MMPs2) and vascular endothelial growth factor (VEGF) proteins on extracellular matrix, fibrous distribution and angiogenetic development in periodontitis caused by nicotine effects on periodontal membrane. MATERIALS AND METHODS: In this experimental study, rats were divided into nicotine and control groups. While the rats in the nicotine group (n = 6) were administered 2 mg/kg nicotine sulphate for 28 days, the animals in the control group (n = 6) were only administered 1.5 mL physiologic saline solution subcutaneously for 28 days. RESULTS: Histological sections were prepared and immunohistochemically stained for MMP2 and VEGF. The sections stained with Trichrome-Masson were observed under light microscope. VEGF and MMP2 immunoreactivity of periodontal gingiva and dentin was assessed by immunohistochemical staining. CONCLUSIONS: Nicotine reduces MMP production, disrupts collagen synthesis and causes periodontitis. We observed that nicotine increases periodontitis by disrupting periodontal membrane and prevents tooth to anchor in dental alveoli by disrupting epithelial structure.” (Deveci et al. 2018).

“The current study analyzed the effects of environmental enrichment versus isolation housing on the behavioral sensitization to nicotine in the neonatal quinpirole (NQ; dopamine D2-like agonist) model of dopamine D2 receptor supersensitivity, a rodent model of schizophrenia. NQ treatment in rats increases dopamine D2 receptor sensitivity throughout the animal's lifetime, consistent with schizophrenia. Animals were administered NQ (1 mg/kg) or saline (NS) from postnatal day (P)1 to P21, weaned, and immediately placed into enriched housing or isolated in wire cages throughout the experiment. Rats were behaviorally sensitized to nicotine (0.5 mg/kg base) or saline every consecutive day from P38 to P45, and brain tissue was harvested at P46. Results revealed that neither housing condition reduced nicotine sensitization in NQ rats, whereas enrichment reduced sensitization to nicotine in NS-treated animals. The nucleus accumbens (NAcc) was analyzed for glial cell line-derived neurotrophic factor (GDNF), a neurotrophin important in dopamine plasticity. Results were complex, and revealed that NAcc GDNF was increased in animals given nicotine, regardless of housing condition. Further, enrichment increased GDNF in NQ rats regardless of adolescent drug treatment and in NS-treated rats given nicotine, but did not increase GDNF in NS-treated controls compared to the isolated housing condition. This study demonstrates that environmental experience has a prominent impact on the behavioral and the neural plasticity NAcc response to nicotine in adolescence.” (Brown et al. 2018).

“BACKGROUND: Cigarette smoking by pregnant women is associated with a significant increase in the risk for cognitive disorders in their children. Preclinical models confirm this risk by showing that exposure of the developing brain to nicotine produces adverse behavioral outcomes. Here we describe behavioral phenotypes resulting from perinatal nicotine exposure in a mouse model, and discuss our findings in the context of findings from previously published studies using preclinical models of developmental nicotine exposure. METHODOLOGY/PRINCIPAL FINDINGS: Female C57Bl/6 mice received drinking water containing nicotine (100μg/ml) + saccharin (2%) starting 3 weeks prior to breeding and continuing throughout pregnancy, and until 3 weeks postpartum. Over the same period, female mice in two control groups received drinking water containing saccharin (2%) or plain drinking water. Offspring from each group were weaned at 3-weeks of age and subjected to behavioral analyses at 3 months of age. We examined spontaneous locomotor activity, anxiety-like behavior, spatial working memory, object based attention, recognition memory and impulsive-like behavior. We found significant deficits in attention and working memory only in male mice, and no significant changes in the other behavioral phenotypes in male or female mice. Exposure to saccharin alone did not produce significant changes in either sex. CONCLUSION/SIGNIFICANCE: The perinatal nicotine exposure produced significant deficits in attention and working memory in a sex-dependent manner in that the male but not female offspring displayed these behaviors. These behavioral phenotypes are associated with attention deficit hyperactivity disorder (ADHD) and have been reported in other studies that used pre- or perinatal nicotine exposure. Therefore, we suggest that preclinical models of developmental nicotine exposure could be useful tools for modeling ADHD and related disorders.” (Zhang L et al. 2018).

“Nicotine, an nAChR agonist, shows prominent anti-inflammatory properties, and some studies have illustrated its suppressive effects on inflammation. Here, we have examined whether nicotine as a medicine may have beneficial effects on the treatment of asthma in a mouse model of allergic asthma. BALB/c mice were sensitized with OVA and alum. Two weeks later, the mice received nicotine with concentrations of 1 and 10 mg/kg three times every other day. After 10 days, the mice were challenged with OVA (5%) using an ultrasonic nebulizer and died the next day. Our results showed that the administration of nicotine reduced lung-tissue inflammation, the number of eosinophils in bronchoalveolar fluid, allergen-specific IgE and IL-4 production, while it increased the TGF-β/IL-4 ratio and the number of Treg cells. Our results showed that nicotine applies its suppressive effects in a dose-dependent manner: administration of 10 mg/kg of nicotine showed more suppressive effects than 1 mg/kg. Such data suggested that nicotine might be a good candidate to be used as a medicine in the treatment of allergic asthma by decreasing allergic inflammation severity and potentiating Treg cells proliferation against the allergen.” (Mazloomi et al. 2018).

“AIMS: Bony complications of diabetes mellitus (DM) are still insufficiently understood. Our aims were to analyze the individual and combined effects of chronic hyperglycemia and nicotine exposure on the femoral trabecular and cortical microarchitecture on a rat experimental model. MAIN METHODS: The micro-computed tomography based bone microstructural evaluation was performed on male Wistar rats divided into four groups: control (n = 7), experimentally-induced DM (n = 8), chronically exposed to nicotine (n = 9) and the DM group exposed chronically to nicotine (n = 9). KEY FINDINGS: Chronic hyperglycemia caused mild trabecular deterioration; yet, the combination of hyperglycemia and nicotine exposure showed more deleterious effects on the trabecular bone. Namely, the DM + nicotine group had significantly lower bone volume fraction, fewer and more rod-like shaped trabeculae, along with higher trabecular separation and lower connectivity than the control group (p < 0.05). Nicotine alone did not show any significant deterioration compared to the control group. DM and DM + nicotine groups had lower cortical porosity than control and nicotine groups (p < 0.05). Cortical thickness did not show any significant intergroup differences, whereas bone perimeter and the mean polar moment of inertia were reduced in DM + nicotine group. SIGNIFICANCE: Mild effects of chronic hyperglycemia on bone structure were accentuated by the chronic nicotine exposure, although nicotine alone did not cause any significant bone changes. That suggests a synergistic effect of hyperglycemia and nicotine on bone deterioration and increased propensity to fracture. Indeed, better understanding of risk factors driving bone structural deterioration is a precondition to limit the complications associated with DM.” (Milovanovic et al. 2018).

“Impaired placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) activity which inactivates maternal glucocorticoids is associated with poor fetal growth and a higher risk of chronic diseases in adulthood. This study aimed to elucidate the epigenetically regulatory mechanism of nicotine on placental 11β-HSD2 expression. Pregnant Wistar rats were administered 1.0 mg/kg nicotine subcutaneously twice a day from gestational day 9 to 20. The results showed that prenatal nicotine exposure increased corticosterone levels in the placenta and fetal serum, disrupted placental morphology and endocrine function, and reduced fetal bodyweight. Meanwhile, histone modification abnormalities (decreased acetylation and increased di-methylation of histone 3 Lysine 9) on the HSD11B2 promoter and lower-expression of 11β-HSD2 were observed. Furthermore, the expression of nicotinic acetylcholine receptor (nAChR) α4/β2, the phosphorylation of extracellular regulated kinase 1/2 (ERK1/2) and Ets-like protein-1 (Elk-1), and the expression of early growth response-1 (Egr-1) were increased in the nicotine groups. In human BeWo cells, nicotine decreased 11β-HSD2 expression, increased nAChRα9 expression, and activated ERK1/2/Elk-1/Egr-1 signaling in the concentration (0.1-10 μM)-dependent manner. Antagonism of nAChRs, inhibition of ERK1/2 and Egr-1 knockdown by siRNA were able to block/abrogate the effects of nicotine on histone modification and expression of 11β-HSD2. Taken together, nicotine can impair placental structure and function, and induce fetal developmental toxicity. The underlying mechanism involves histone modifications and down-regulation of 11β-HSD2 through nAChRs/ERK1/2/Elk-1/Egr-1 signaling, which increases active glucocorticoids levels in the placenta and fetus, and eventually inhibits the fetal development.” (Zhou J et al. 2018).

“With the increasing popularity of E-cigarettes, chronic exposure to nicotine (NIC) is emerging as a novel risk factor for the kidney. NIC increases oxidative stress in the kidneys, which impairs the viability and function of renal tubular and endothelial cells, alters renal hemodynamics, and compromises overall kidney function. Moreover, long-term NIC exposure increases the risk of development and progression of chronic kidney diseases and may escalate the impact of coexisting morbidities such as obesity-associated renal disease, hypertension, renal transplant status, or the toxicity of various anticancer agents. In this review, we summarize experimental findings describing increased renal risk of chronic NIC exposure and explore therapeutic interventions to alleviate adverse effects of NIC.” (Arany et al 2018b).

“In a Sprague-Dawley rat study, animals were given an average of 1.14 or 4.56 mg/kg/day of nicotine alkaloid in the drinking water for 34 weeks. After the end of the treatment, half of each group and an equal number of untreated controls were then exposed to 6% oxygen for 12 hours. Mortality, gross cardiac lesions, hematocrits, and activity of several heart enzymes, selected as potential indices of early injury were afterwards examined at several intervals. The only effects reported in nicotine-only-treated animals were a statistically significant increase in the activity of isocitric dehydrogenase and acid phosphatase and a statistically significant decrease in the activity of β-glucoronidase at the high dose.” (Wenzel and Richard 1970).

“To assess the effects of long-term treatment of nicotine on several behavior measures, including locomotor activity, exploratory efficiency habituations, short-term and long-term memory, forty five young (5 months) and 45 old (22 months) female Sprague-Dawley rats. Animals (n=15/group) were given nicotine (as nicotine tartrate) via the drinking water at concentrations of 0, 20 or 50 mg/L for 131 days. Nicotine intake consistently increased locomotor activity and decreased body weight and fluid intake throughout the treatment period in both young and old rats. Only small effects on exploratory efficiency could be observed in young rats. Nicotine did not affect habituation and memory tasks.” (Welzl et al. 1988).

Yuen et al., (1995) studied the direct effect of nicotine on the liver, both when given alone and when combined with carbon tetrachloride in female Sprague-Dawley rats. Groups of rats received 54 and 108 µmol/L of nicotine in drinking water for 10 days (equivalent to 1.25 and 2.5 mg/kg/bw). A sub-group was also given a single subcutaneous injection of 6g/kg of carbon tetrachloride shortly before the animals were killed. Additionally, another sub-group of 10 days pregnant rats underwent the same treatment. Histology demonstrated a significant hepatotoxic effect in the group receiving 108 µmol/L of nicotine when compared with the control group in a form of fatty change, focal or confluent necrosis and dark-cell change. Nicotine also aggravated the hepatoxicity of caron tetrachloride. Animals receiving the lower nicotine dose did not show any statistically significant histopathological changes when compared with the control group. The effects in pregnant rats were less severe. The no observed adverse effect level (NOAEL) was identified as 1.25 mg/kg/day.

“The effects of nicotine and its withdrawal on locomotor activity and brain monoamines were studied in male NMRI mice. Animals were given increasing concentrations of nicotine in drinking water for 50 days, equivalent to 60-65 mg/kg bw/day from the 3rd week until the end of the treatment. Locomotor activity was significantly increased on the 50th day of nicotine administration compared to the control animals. However, no difference was observed 12-14 hours after the exposure cessation. Additionally, concentrations of several brain monoamines were elevated on the 50th day, but at 23-25 hours after withdrawal, only hypothalamic noradrenaline concentration was still elevated.” (Gäddnäs et al. 2000).

Recent 90-day study in rats (unpublished data) demonstrated that inhalation of e-vapor (3.2-9.6 mg/kg/day of aerosol mass) containing 1.5% of nicotine did not induce any adverse histopathological changes in the lungs of treated rats compared with control animals (Werley M et al., 2014).

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| **Spec. Sci. Name**      **Spec. Common Name** | | **Media Type** | **Test Loc.** | **Exp. Type** | **Dose #** | **Endpoint** | **Effect** |  | **Effect Meas.** | **Chem. Anal.** | **Conc. Type**  **Dose** | **Obs. Dur. (Days)** |
|  | **CAS #/Chemical: 54115 3-[(2S)-1-Methyl-2-pyrrolidinyl]pyridine** | | | | | | | | | | | |
|  | **Mammals** | | | | | | | | | | | |
| Mus musculus | | NONE | LAB | DR | 11 | IC50 | AVO |  | WATR | U | F | 10 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 42.8 ug/ml |  |
| Mus musculus | | NONE | LAB | DR | 11 | IC50 | AVO |  | WATR | U | F | 10 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 47.3 ug/ml |  |
| Mus musculus | | NONE | LAB | DR | 11 | IC50 | AVO |  | WATR | U | F | 10 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 72.0 ug/ml |  |
| Mus musculus | | NONE | LAB | DR | 11 | IC50 | AVO |  | WATR | U | F | 10 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 76.9 ug/ml |  |
| Mus musculus | | NONE | LAB | DR | 11 | IC50 | AVO |  | WATR | U | F | 10 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 40.2 ug/ml |  |
| Mus musculus | | NONE | LAB | DR | 11 | IC50 | AVO |  | WATR | U | F | 10 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 46.3 ug/ml |  |
| Mus musculus | | NONE | LAB | DR | 11 | IC50 | AVO |  | WATR | U | F | 10 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 114.1 ug/ml |  |
| Mus musculus | | NONE | LAB | DR | 11 | IC50 | AVO |  | WATR | U | F | 10 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 113.3 ug/ml |  |
| Mus musculus | | NONE | LAB | DR | 11 | IC50 | AVO |  | WATR | U | F | 10 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 89.7 ug/ml |  |
| Mus musculus | | NONE | LAB | DR | 11 | IC50 | AVO |  | WATR | U | F | 10 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 98.7 ug/ml |  |
| Mus musculus | | NONE | LAB | DR | 11 | IC50 | AVO |  | WATR | U | F | 10 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 37.4 ug/ml |  |
| Mus musculus | | NONE | LAB | DR | 11 | IC50 | AVO |  | WATR | U | F | 10 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 46.1 ug/ml |  |
| Mus musculus | |  | LAB | DR | 4 | LOEL | AVO |  | CHEM | M | A | 30 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 126.8 ug/ml |  |
| Mus musculus | | NONE | LAB | DR | 2 | LOEL | BCM |  | HVLA | U | F | 50 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | <= 500 ug/ml |  |
| Mus musculus | | NONE | LAB | SC | 3 | LOEL | BCM |  | CHOL | U | F | 10 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.35 mg/100g bw/d |  |
| Mus musculus | | NONE | LAB | MU | 2 | LOEL | BCM |  | HVLA | U | NC | 7 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | > 40 ul |  |
| Mus musculus | | NONE | LAB | MU | 2 | LOEL | BCM |  | 3MTA | U | NC | 7 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | > 40 ul |  |
| Mus musculus | | NONE | LAB | MU | 2 | LOEL | BCM |  | 3MTA | U | NC | 7 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | > 40 ul |  |
| Mus musculus | | NONE | LAB | DR | 2 | LOEL | BCM |  | ASHC | U | F | 56 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 2 ug/d/org |  |
| Cavia porcellus | | NONE | LAB | OP | 2 | LOEL | BCM |  | SAME | U | F | 21 d |
| Guinea Pig | |  |  |  |  |  |  |  |  |  | 600 ug/h |  |
| Mus musculus | | NONE | LAB | DT | 2 | LOEL | BCM |  | DOPC | U | F | 50 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 500 ug/ml |  |
| Mus musculus | | NONE | LAB | IP | 4 | LOEL | BCM |  | CHOL | U | F | 15 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.2 mg/100g bw/d |  |
| Cavia porcellus | | NONE | LAB | OP | 2 | LOEL | BCM |  | SAHC | U | F | 21 d |
| Guinea Pig | |  |  |  |  |  |  |  |  |  | 600 ug/h |  |
| Cavia porcellus | | NONE | LAB | SC | 2 | LOEL | BEH |  | HMVT | U | F | 10 d |
| Guinea Pig | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg |  |
| Mus musculus | |  | LAB | DR | 2 | LOEL | BEH |  | LOCO | U | F | 3 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 100 ug/ml |  |
| Mus musculus | | NONE | LAB | SC | 2 | LOEL | BEH |  | GBHV | U | F | 45 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.5 mg/kg |  |
| Mus musculus | | NONE | LAB | DT | 2 | LOEL | BEH |  | ACTV | U | F | 50 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 500 ug/ml |  |
| Mesocricetus auratus | | NONE | LAB | TP | 2 | LOEL | CEL |  | RSBC | U | F | 28 d |
| Golden Hamster | |  |  |  |  |  |  |  |  |  | 0.5 ug/ml |  |
| Mus musculus | | NONE | LAB | SC | 2 | LOEL | CEL |  | RSBC | U | F | 15 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Mus musculus | |  | LAB | DR | 2 | LOEL | CEL |  | RSBC | U | F | 30 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 200 ug/ml |  |
| Mus musculus | | NONE | LAB | DR | 2 | LOEL | DVP |  | WGHT | U | F | 66 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 1 mg/L |  |
| Mus musculus | | NONE | LAB | SC | 2 | LOEL | DVP |  | FURR | U | F | 19 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.5 mg/kg |  |
| Mus musculus | | CUL | LAB | CM | 3 | LOEL | DVP |  | BSCY | U | A | 4.063 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 500 uM |  |
| Mus musculus | | CUL | LAB | CM | 2 | LOEL | DVP |  | BSCY | U | A | 4 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 500/ (NR/-NR/) uM |  |
| Mus musculus | | CUL | LAB | CM | 2 | LOEL | DVP |  | BSCY | U | A | 4.063 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 500/ (NR/-NR/) uM |  |
| Mus musculus | | CUL | LAB | CM | 2 | LOEL | DVP |  | BSCY | U | A | 4 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 500/ (NR/-NR/) uM |  |
| Mus musculus | | NONE | LAB | SC | 2 | LOEL | GEN |  | MRNA | U | F | 7 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Mus musculus | | NONE | LAB | DR | 3 | LOEL | GRO |  | WGHT | U | F | 14 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 60 ug/ml |  |
| Mus musculus | | NONE | LAB | SC | 2 | LOEL | GRO |  | WGHT | U | F | 15 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Mus musculus | | NONE | LAB | DR | 2 | LOEL | GRO |  | WGHT | U | F | 56 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 2 ug/d/org |  |
| Mus musculus | | NONE | LAB | SC | 2 | LOEL | GRO |  | WGHT | U | F | 23 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.5 mg/kg |  |
| Mus musculus | | NONE | LAB | IP | 4 | LOEL | GRO |  | DMTR | U | F | 15 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.6 mg/100g bw/d |  |
| Mesocricetus auratus | | NONE | LAB | TP | 2 | LOEL | HIS |  | LESI | U | F | 84 d |
| Golden Hamster | |  |  |  |  |  |  |  |  |  | 50 ul |  |
| Mus musculus | | NONE | LAB | DR | 2 | LOEL | HIS |  | BODS | U | F | 66 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 1 mg/L |  |
| Mus musculus | | NONE | LAB | DR | 2 | LOEL | HIS |  | BODS | U | F | 41 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 1 mg/L |  |
| Mus musculus | | NONE | LAB | DR | 2 | LOEL | HRM |  | DOPA | U | F | 50 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | <= 500 ug/ml |  |
| Mus musculus | | NONE | LAB | SC | 2 | LOEL | HRM |  | NORE | U | F | 15 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Mus musculus | | NONE | LAB | DT | 2 | LOEL | HRM |  | DOPA | U | F | 50 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 500 ug/ml |  |
| Mus musculus | | NONE | LAB | DR | 2 | LOEL | IMM |  | MCPG | U | F | 66 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 1 mg/L |  |
| Mus musculus | | NONE | LAB | LC | 2 | LOEL | IMM |  | MCPG | U | F | 30 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 1 mg/L |  |
| Mus musculus | | NONE | LAB | DR | 2 | LOEL | IMM |  | MCPG | U | F | 66 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 1 mg/L |  |
| Mesocricetus auratus | | NONE | LAB | TP | 2 | LOEL | INJ |  | TUMR | U | F | 84 d |
| Golden Hamster | |  |  |  |  |  |  |  |  |  | 50 ul |  |
| Mus musculus | | NONE | LAB | DR | 3 | LOEL | MPH |  | WGHT | U | F | 14 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 100 ug/ml |  |
| Mus musculus | | NONE | LAB | SC | 3 | LOEL | MPH |  | SMIX | U | F | 10 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.35 mg/100g bw/d |  |
| Mus musculus | | NONE | LAB | DR | 2 | LOEL | MPH |  | DNSY | U | F | 56 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 2 ug/d/org |  |
| Mus musculus | | NONE | LAB | IP | 4 | LOEL | MPH |  | SMIX | U | F | 15 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.2 mg/100g bw/d |  |
| Mus musculus | | NONE | LAB | IP | 4 | LOEL | PHY |  | EECG | U | F | 14 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.19 umol/kg |  |
| Mus musculus | | NONE | LAB | MU | 2 | LOEL | PHY |  | THRG | U | NC | 7 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 40 ul |  |
| Sus sp. | | NONE | LAB | IM | 2 | LOEL | PHY |  | CRAT | U | F | 126 d |
| Pig | |  |  |  |  |  |  |  |  |  | 17 ug/ml |  |
| Mus musculus | | NONE | LAB | SC | 2 | LOEL | PHY |  | OXYG | U | F | 15 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Mus musculus | | NONE | LAB | SC | 3 | LOEL | REP |  | SPMC | U | F | 10 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.15 mg/100g bw/d |  |
| Mus musculus | | NONE | LAB | IP | 4 | LOEL | REP |  | SPMC | U | F | 15 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.2 mg/100g bw/d |  |
| Mus musculus | |  | LAB | DR | 4 | NOEL | AVO |  | CHEM | M | A | 30 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 68.49 ug/ml |  |
| Mus musculus | | NONE | LAB | IP | 2 | NOEL | BCM |  | WTCO | U | F | 5 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 1.3 mg/kg |  |
| Mus musculus | | NONE | LAB | DR | 3 | NOEL | BCM |  | WTCO | U | F | 14 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 100 ug/ml |  |
| Mus musculus | | NONE | LAB | SC | 3 | NOEL | BCM |  | CHOL | U | F | 10 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.15 mg/100g bw/d |  |
| Mus musculus | | NONE | LAB | MU | 2 | NOEL | BCM |  | 3MTA | U | NC | 7 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 40 ul |  |
| Mus musculus | | NONE | LAB | MU | 2 | NOEL | BCM |  | 3MTA | U | NC | 7 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 40 ul |  |
| Mus musculus | | NONE | LAB | MU | 2 | NOEL | BCM |  | 3MTA | U | NC | 7 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 40 ul |  |
| Mus musculus | |  | LAB | MU | 2 | NOEL | BCM |  | HVLA | U | F | 49 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Mus musculus | |  | LAB | DR | 2 | NOEL | BCM |  | HVLA | U | F | 49 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | <= 500 ug/ml |  |
| Mus musculus | | NONE | LAB | SC | 2 | NOEL | BCM |  | GBCM | U | F | 3 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Cavia porcellus | | NONE | LAB | OP | 2 | NOEL | BCM |  | SAHC | U | F | 21 d |
| Guinea Pig | |  |  |  |  |  |  |  |  |  | 600 ug/h |  |
| Cavia porcellus | | NONE | LAB | OP | 2 | NOEL | BCM |  | SAME | U | F | 21 d |
| Guinea Pig | |  |  |  |  |  |  |  |  |  | 600 ug/h |  |
| Mus musculus | | NONE | LAB | IV | 2 | NOEL | BEH |  | NMVM | U | F | 7 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 4 mg/kg/h |  |
| Mus musculus | |  | LAB | MU | 4 | NOEL | BEH |  | LOCO | M | F | 30 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 200 ug/ml |  |
| Mus musculus | | NONE | LAB | DR | 2 | NOEL | DVP |  | WGHT | U | F | 66 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 1 mg/L |  |
| Mus musculus | | NONE | LAB | LC | 2 | NOEL | DVP |  | WGHT | U | F | 30 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 1 mg/L |  |
| Mus musculus | | NONE | LAB | DR | 3 | NOEL | ENZ |  | ACHE | U | F | 14 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 100 ug/ml |  |
| Mus musculus | | NONE | LAB | SC | 2 | NOEL | FDB |  | FCNS | U | F | 15 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Mus musculus | |  | LAB | DR | 4 | NOEL | FDB |  | FCNS | M | A | 30 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 126.8 ug/ml |  |
| Mus musculus | | NONE | LAB | SC | 2 | NOEL | GEN |  | MRNA | U | F | 3 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Mus musculus | | CUL | LAB | CM | 3 | NOEL | GEN |  | APOP | U | A | 4.063 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 5 mM |  |
| Mus musculus | | NONE | LAB | DT | 2 | NOEL | GRO |  | WGHT | U | F | 32 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.01% |  |
| Mus musculus | | NONE | LAB | IP | 2 | NOEL | GRO |  | WGHT | U | F | 5 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 1.3 mg/kg |  |
| Mus musculus | |  | LAB | DR | 4 | NOEL | GRO |  | GAIN | M | A | 30 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 126.8 ug/ml |  |
| Mus musculus | | NONE | LAB | IP | 4 | NOEL | GRO |  | DMTR | U | F | 15 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.4 mg/100g bw/d |  |
| Mus musculus | | NONE | LAB | LC | 2 | NOEL | HIS |  | BODS | U | F | 30 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 1 mg/L |  |
| Mesocricetus auratus | | NONE | LAB | TP | 2 | NOEL | HRM |  | GHRM | U | F | 28 d |
| Golden Hamster | |  |  |  |  |  |  |  |  |  | 0.5 ug/ml |  |
| Mus musculus | | NONE | LAB | DT | 2 | NOEL | INJ |  | DAMG | U | F | 32 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.01% |  |
| Mus musculus | | NONE | LAB | DT | 2 | NOEL | MPH |  | WGHT | U | F | 4 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.01% |  |
| Mus musculus | | NONE | LAB | DR | 3 | NOEL | MPH |  | WGHT | U | F | 14 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 60 ug/ml |  |
| Mus musculus | | NONE | LAB | IP | 2 | NOEL | MPH |  | WGHT | U | F | 5 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 1.3 mg/kg |  |
| Mus musculus | | NONE | LAB | SC | 3 | NOEL | MPH |  | SMIX | U | F | 10 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 0.15 mg/100g bw/d |  |
| Mus musculus | | NONE | LAB | DR | 2 | NOEL | MPH |  | VOLU | U | F | 66 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 1 mg/L |  |
| Mus musculus | | NONE | LAB | LC | 2 | NOEL | MPH |  | VOLU | U | F | 30 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 1 mg/L |  |
| Mus musculus | | NONE | LAB | DR | 2 | NOEL | MPH |  | VOLU | U | F | 66 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 1 mg/L |  |
| Mus musculus | |  | LAB | MU | 4 | NOEL | PHY |  | BTMP | U | F | 30 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 200 ug/ml |  |
| Mus musculus | |  | LAB | DR | 2 | NOEL | PHY |  | BTMP | U | F | 3 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 100 ug/ml |  |
| Mus musculus | |  | LAB | DR | 2 | NOEL | PHY |  | BTMP | U | F | 49 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | <= 500 ug/ml |  |
| Mus musculus | | NONE | LAB | IP | 2 | NOEL | REP |  | PROG | U | F | 5 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 1.3 mg/kg |  |
| Mus musculus | | NONE | LAB | DR | 3 | NOEL | REP |  | PROG | U | F | 14 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 100 ug/ml |  |
| Mus sp. | |  | LAB | GV | 2 |  | GRO |  | WGHT | U | NC | 14 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | (0-35) mg/kg org/d |  |
| Mus sp. | |  | LAB | GV | 2 |  | MOR |  | GMOR/ | U | NC | 14 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | (0-35) mg/kg org/d |  |
| Mus musculus | | CUL | LAB | CM | 3 |  | MOR |  | MORT | U | A | 4.063 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | 5 mM |  |
| Mus sp. | |  | LAB | GV | 2 |  | REP |  | GREP/ | U | NC | 14 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | (0-35) mg/kg org/d |  |
| Mus musculus | | CUL | LAB | CM | 3 |  | REP |  | MOTL | U | A | 4.063 d |
| House Mouse | |  |  |  |  |  |  |  |  |  | (500-5000) uM |  |
|  | **Mammals; Standard Test Species** | | | | | | | | | | | |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEC | FDB |  | FCNS |  | NC | 70 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEC | FDB |  | FCNS |  | NC | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | LOEC | FDB |  | FCNS | U | F | 33 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 4 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | LOEC | FDB |  | WCON | U | F | 33 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEC | GRO |  | WGHT |  | NC | 70 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEC | GRO |  | WGHT |  | NC | 84 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEC | GRO |  | WGHT |  | NC | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEC | GRO |  | WGHT |  | NC | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | LOEC | GRO |  | GAIN | U | F | 33 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | LOEC | GRO |  | WGHT | U | F | 33 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | LOEC | GRO |  | WGHT | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEC | HRM |  | TSHT | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEC | HRM |  | TRII | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEC | HRM |  | THYR | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | LOEC | MPH |  | WGHT | U | F | 6 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 9.6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | LOEC | MPH |  | WGHT | U | F | 6 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 9.6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | LOEC | PHY |  | BDVL | U | F | 6 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2.4 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | LOEC | PHY |  | FLUX | U | F | 6 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2.4 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEC | PHY |  | RESP | U | F | 17 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEC | PHY |  | RESP | U | F | 26 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEC | PHY |  | RESP | U | F | 18 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEC | PHY |  | RESP | U | F | 34 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | ACC |  | RSDE | U | F | 50 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 ug/ml |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | BCM |  | GBCM | U | F | 4 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 4 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | BCM |  | GBCM | U | F | 4 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 8 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | BCM |  | THBA | U | F | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | IJ | 2 | LOEL | BCM |  | CUCO | U | F | 84 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | BCM |  | CHOL | U | F | 35 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 5 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | BCM |  | TRYS | U | F | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 mg/org |  |
| Rattus norvegicus | | NONE | LAB | SC | 6 | LOEL | BCM |  | PHST | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.2 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | BCM |  | PHST | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.6 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 3 | LOEL | BCM |  | PRCO | U | F | 24 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.02 mg/ml |  |
| Rattus norvegicus | | NONE | LAB | IP | 2 | LOEL | BCM |  | PRCO | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.04 mg/100g bw |  |
| Rattus norvegicus | | NONE | LAB | IJ | 2 | LOEL | BCM |  | 5HAA | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.8 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | GV | 2 | LOEL | BCM |  | PRCO | U | F | 122 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 20 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | IP | 3 | LOEL | BCM |  | LACT | U | F | 35 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.25 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | BCM |  | ACHL | U | A | 10 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.6 uM/kg |  |
| Rattus norvegicus | | NONE | LAB | DT | 2 | LOEL | BCM |  | FFTA | U | A | 90 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.5 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | IP | 3 | LOEL | BCM |  | GLYC | U | F | 20 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | IP | 2 | LOEL | BCM |  | CHOL | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.3 mg/100g bw/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 3 | LOEL | BCM |  | GLUC | U | A | 112 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 mg/L |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | BCM |  | 5HAA | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 3 | LOEL | BCM |  | DOPC | U | F | 77 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | BCM |  | PRCO | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | BCM |  | PRCO | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | BCM |  | PRCO | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | BCM |  | PRCO | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | DT | 2 | LOEL | BCM |  | LACT | U | F | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 4 | LOEL | BCM |  | PRCO | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | BCM |  | GLUC | U | F | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.77 mM |  |
| Rattus norvegicus | | NONE | LAB | DR | 3 | LOEL | BCM |  | GLUC | U | F | 112 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.31 mM |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | BCM |  | GLUC | U | F | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.77 mM |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | BCM |  | TRYP | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | BCM |  | 5HAA | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | LOEL | BEH |  | STPY | U | F | 33 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | IP | 3 | LOEL | BEH |  | ACTV | U | F | 6 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | IP | 2 | LOEL | BEH |  | ACTV | U | F | 13 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.32 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | BEH |  | ACTV | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | BEH |  | VMVT | U | F | 102 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | BEH |  | ACTP | U | F | 15 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | BEH |  | ACTP | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.45 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | BEH |  | HMVT | U | F | 11 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | BEH |  | HMVT | U | F | 11 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 3 | LOEL | BEH |  | GBHV | U | F | 3 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.5 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | IJ | 4 | LOEL | BEH |  | ACTV | U | F | 3 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 10 ug/org |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | BEH |  | ACTV | U | F | 9 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | BEH |  | HMVT | U | F | 5 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.5 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | DR | 3 | LOEL | BEH |  | NMVM | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 mg/L |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | BEH |  | NMVM | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 mg/L |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | BEH |  | NMVM | U | F | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 mg/L |  |
| Rattus norvegicus | | NONE | LAB | DR | 4 | LOEL | BEH |  | DUMV | M | A | 50 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 ug/ml |  |
| Rattus norvegicus | | NONE | LAB | IJ | 2 | LOEL | BEH |  | ACTV | U | A | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.4 mg/d |  |
| Rattus norvegicus | | NONE | LAB | DM | 2 | LOEL | BEH |  | NMVM | U | F | 3 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 5.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | BEH |  | REST | U | A | 11 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.4 mg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | CEL |  | DMTR | U | F | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 5 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | CEL |  | RSBC | U | F | 12 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | IP | 2 | LOEL | CEL |  | DNSY | U | F | 56 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2.5 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | MU | 2 | LOEL | CEL |  | MUCR | U | F | 13 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 100 mg/L |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | CEL |  | RSBC | U | NC | 119 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | CEL |  | RSBC | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 4 | LOEL | CEL |  | NCEL | U | A | 4.083 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 5.0 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | CEL |  | NCEL | U | A | 3.083 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 5 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | CEL |  | NCEL | U | A | 4.083 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 5 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | CEL |  | RSBC | U | A | 10 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.6 uM/kg |  |
| Rattus norvegicus | | NONE | LAB | IP | 2 | LOEL | CEL |  | NCEL | U | F | 6 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | CEL |  | PKNJ | U | F | 35 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 mg/L |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | CEL |  | RSBC | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 100 mg/L |  |
| Rattus norvegicus | | NONE | LAB | GV | 2 | LOEL | CEL |  | HGHT | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.3 mg/100g bw/d |  |
| Rattus norvegicus | | NONE | LAB | IP | 2 | LOEL | CEL |  | HGHT | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.3 mg/100g bw/d |  |
| Rattus norvegicus | | CUL | LAB | CM | 4 | LOEL | CEL |  | NCEL | U | F | 2 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1 uM |  |
| Rattus norvegicus | | NONE | LAB | DT | 4 | LOEL | CEL |  | DNSY | U | F | 58 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.01% |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | DVP |  | DVLP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 150 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 150 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 150 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 150 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 150 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 150 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 150 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 150 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 150 ug/h |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | ENZ |  | GENZ | U | F | 4 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 4 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | ENZ |  | GENZ | U | F | 4 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 8 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | ENZ |  | UDPT | U | A | 20 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.0 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | ENZ |  | CRLP | U | F | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | ENZ |  | GLPX | U | F | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | IJ | 2 | LOEL | ENZ |  | CRLP | U | F | 84 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | ENZ |  | TPSY | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 mg/org |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | ENZ |  | TPSY | U | F | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 mg/org |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | ENZ |  | MALE | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.6 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | ENZ |  | TBHY | U | F | 4 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.03 mg/ml |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | ENZ |  | ORDC | U | F | 13 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | IP | 2 | LOEL | ENZ |  | ALPH | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.04 mg/100g bw |  |
| Rattus norvegicus | | NONE | LAB | GV | 2 | LOEL | ENZ |  | TPSY | U | F | 122 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 20 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DT | 2 | LOEL | ENZ |  | CTLS | U | A | 90 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.5 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | ENZ |  | TSHX | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | GV | 2 | LOEL | ENZ |  | ALPH | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.3 mg/100g bw/d |  |
| Rattus norvegicus | | NONE | LAB | IP | 2 | LOEL | ENZ |  | ACPH | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.3 mg/100g bw/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 3 | LOEL | ENZ |  | CATP | M | A | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.172 mg/100g bw |  |
| Rattus norvegicus | | NONE | LAB | DR | 3 | LOEL | ENZ |  | GENZ | U | A | 112 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 mg/L |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | ENZ |  | TSHX | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | ENZ |  | TSHX | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | ENZ |  | TSHX | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | ENZ |  | TSHX | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | DT | 2 | LOEL | ENZ |  | PPHL | U | F | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | LOEL | FDB |  | FCNS | U | F | 19 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 4 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | FDB |  | FCNS |  | NC | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 4 | LOEL | FDB |  | WCON | M | A | 50 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 31.8 ug/ml |  |
| Rattus norvegicus | | NONE | LAB | SC | 4 | LOEL | FDB |  | FCNS | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.6 mg/kg bdwt |  |
| Rattus norvegicus | | NONE | LAB | DR | 3 | LOEL | FDB |  | WCON | M | A | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.172 mg/100g bw |  |
| Rattus norvegicus | | NONE | LAB | DR | 3 | LOEL | FDB |  | WCON | U | A | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 mg/L |  |
| Rattus norvegicus | | NONE | LAB | IJ | 2 | LOEL | FDB |  | FCNS | U | A | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.4 mg/d |  |
| Rattus norvegicus | | NONE | LAB | DM | 2 | LOEL | FDB |  | WCON | U | F | 4 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 5 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | FDB |  | WCON | U | F | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.77 mM |  |
| Rattus norvegicus | | NONE | LAB | DR | 3 | LOEL | FDB |  | FCNS | U | F | 112 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.31 mM |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | FDB |  | FCNS | U | F | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.77 mM |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | FDB |  | FCNS | U | A | 11 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.4 mg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | GEN |  | MRNA | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 mg/org |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | GEN |  | MRNA | U | F | 4 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.8 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | GEN |  | RNDN | U | F | 13 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | IP | 2 | LOEL | GEN |  | DNAC | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.04 mg/100g bw |  |
| Rattus norvegicus | | NONE | LAB | GV | 2 | LOEL | GEN |  | DNAC | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.3 mg/100g bw/d |  |
| Rattus norvegicus | | NONE | LAB | IP | 2 | LOEL | GEN |  | DNAC | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.3 mg/100g bw/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | GEN |  | MRNA | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | GEN |  | MRNA | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | GEN |  | MRNA | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | GEN |  | MRNA | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | IP | 2 | LOEL | GEN |  | DNAB | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | LOEL | GRO |  | WGHT | U | F | 24 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.75 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | GRO |  | WGHT | U | F | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | GRO |  | WGHT | U | F | 15 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | LOEL | GRO |  | WGHT | U | F | 19 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg/d |  |
| Rattus norvegicus | | MIX | LAB | DT | 2 | LOEL | GRO |  | WGHT | U | F | 27 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 100 mg/L |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | GRO |  | GAIN | U | F | 4 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.03 mg/ml |  |
| Rattus norvegicus | | NONE | LAB | DR | 3 | LOEL | GRO |  | WGHT | U | F | 75 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2.4 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | GRO |  | WGHT |  | NC | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | IP | 2 | LOEL | GRO |  | GAIN | U | F | 30 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | GV | 2 | LOEL | GRO |  | GAIN | U | F | 122 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 20 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | GRO |  | WGHT | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 100 mg/L |  |
| Rattus norvegicus | | NONE | LAB | SC | 4 | LOEL | GRO |  | GAIN | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.6 mg/kg bdwt |  |
| Rattus norvegicus | | NONE | LAB | DR | 3 | LOEL | GRO |  | GAIN | U | A | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 mg/L |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | GRO |  | WGHT | U | F | 50 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 ug/ml |  |
| Rattus norvegicus | | NONE | LAB | SC | 3 | LOEL | GRO |  | WGHT | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DA | 2 | LOEL | GRO |  | GAIN | U | F | 12 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 35 mg/org |  |
| Rattus norvegicus | | NONE | LAB | SC | 4 | LOEL | GRO |  | GAIN | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 3 | LOEL | GRO |  | WGHT | U | F | 112 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.31 mM |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | GRO |  | WGHT | U | F | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.77 mM |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | GRO |  | GAIN | U | F | 17 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | GV | 2 | LOEL | GRO |  | WGHT | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | GV | 2 | LOEL | GRO |  | WGHT | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | HIS |  | ATRS | U | F | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 5 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | IP | 2 | LOEL | HIS |  | PNCH | U | F | 9 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | IP | 2 | LOEL | HIS |  | GHIS | U | F | 56 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2.5 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | IP | 3 | LOEL | HIS |  | IHGT | U | F | 20 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 5 | LOEL | HIS |  | NCRO | U | F | 35 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | HRM |  | CORT | U | F | 87 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | HRM |  | TSTR | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.6 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | HRM |  | CORT | U | F | 11 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | LC | 2 | LOEL | HRM |  | VASO | U | F | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | LC | 2 | LOEL | HRM |  | VASO | U | F | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | MU | 3 | LOEL | HRM |  | LUTH | U | F | 75 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 4.5 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | MU | 3 | LOEL | HRM |  | LUTH | U | F | 75 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 4.5 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 3 | LOEL | HRM |  | LUTH | U | F | 75 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 4.5 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | LC | 3 | LOEL | HRM |  | LUTH | U | F | 75 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 4.5 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | HRM |  | NORE | U | NC | 49 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | IJ | 2 | LOEL | HRM |  | NORE | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.8 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | HRM |  | CORT | U | F | 11 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.5 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | GV | 2 | LOEL | HRM |  | GHRM | U | F | 122 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 20 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | GHRM | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | GHRM | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | PU | 3 | LOEL | HRM |  | VASO | U | F | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 3 | LOEL | HRM |  | INSL | U | A | 112 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 mg/L |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | HRM |  | SRTN | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 3 | LOEL | HRM |  | INSL | U | F | 112 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.31 mM |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | HRM |  | NORE | U | F | 17 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | HRM |  | 5HST | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | HRM |  | SRTN | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | HRM |  | VASO | U | F | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | HRM |  | VASO | U | F | 29 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | HRM |  | VASO | U | F | 29 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | HRM |  | VASO | U | F | 35 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | HRM |  | VASO | U | F | 35 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | HRM |  | VASO | U | F | 35 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | HRM |  | VASO | U | F | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | HRM |  | VASO | U | F | 56 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 47 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 61 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 61 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 61 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 47 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 61 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 61 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 61 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 47 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 47 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 47 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 47 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 60 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 61 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 61 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | HRM |  | VASO | U | F | 61 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | GE | 2 | LOEL | INJ |  | DAMG | U | F | 22.5 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1 mg/kg bdwt |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | INJ |  | ULCR | U | F | 10 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 500 ug % |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | ITX |  | CONV | U | F | 4 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.5 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | ITX |  | TINT | U | F | 3 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.5 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | MOR |  | MORT | U | F | 17 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | DR | 3 | LOEL | MOR |  | LFSP | U | F | 62.8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 mg/L |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | MOR |  | LFSP | U | F | 74 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 mg/L |  |
| Rattus norvegicus | | NONE | LAB | LC | 2 | LOEL | MPH |  | AREA | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1 mg/kg bdwt |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | MPH |  | WGHT | U | F | 609 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DT | 4 | LOEL | MPH |  | DMTR | U | F | 9 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 10 mg/kg bdwt/d |  |
| Rattus norvegicus | | MIX | LAB | DT | 2 | LOEL | MPH |  | WGHT | U | F | 27 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 100 mg/L |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | MPH |  | WGHT | U | F | 13 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | GV | 2 | LOEL | MPH |  | DMTR | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.04 mg/100g bw |  |
| Rattus norvegicus | | NONE | LAB | IP | 2 | LOEL | MPH |  | DMTR | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.04 mg/100g bw |  |
| Rattus norvegicus | | NONE | LAB | IP | 3 | LOEL | MPH |  | SMIX | U | F | 20 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | IP | 2 | LOEL | MPH |  | WGHT | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.3 mg/100g bw/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | MPH |  | LGTH | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | SC | 4 | LOEL | MPH |  | SMIX | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.5 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | MPH |  | WGHT | U | F | 17 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | MPH |  | DMTR | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | MPH |  | LGTH | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | LOEL | MPH |  | SMTE | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 150 ug/h |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | PHY |  | SYPS | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | PHY |  | BTMP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | GV | 2 | LOEL | PHY |  | VOLU | U | F | 122 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 20 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | LOEL | PHY |  | BDVL | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2.4 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | PHY |  | GPHY | U | F | 50 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 ug/ml |  |
| Rattus norvegicus | | NONE | LAB | IJ | 2 | LOEL | PHY |  | EXCR | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | IJ | 2 | LOEL | PHY |  | NRXN | U | NC | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | PHY |  | BLPR | U | F | 35 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | LOEL | PHY |  | VENT | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DM | 2 | LOEL | PHY |  | EECG | U | F | 45 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 5 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DM | 3 | LOEL | REP |  | NPRG | U | F | 20 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.75 mg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | LOEL | REP |  | PROG | U | F | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 3 | LOEL | REP |  | VAOP | U | F | 75 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2.4 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | LOEL | REP |  | RSEM | U | F | 17 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | IP | 2 | LOEL | REP |  | SPCL | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.04 mg/100g bw |  |
| Rattus norvegicus | |  | LAB | DT | 2 | LOEL | REP |  | MSTR | U | F | 12.99 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.3 mg/100g bw/d |  |
| Rattus norvegicus | | NONE | LAB | IP | 3 | LOEL | REP |  | GFST | U | F | 20 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEC | BEH |  | ACTV | U | F | 33 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEC | BEH |  | ACTV | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEC | BEH |  | STPY | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEC | BEH |  | ACTV | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEC | BEH |  | ACTV | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEC | BEH |  | ACTV | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEC | BEH |  | ACTV | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | CEL |  | RSBC | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | ENZ |  | TSHX | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | ENZ |  | TSHX | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | ENZ |  | TSHX | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | ENZ |  | TSHX | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | ENZ |  | ITMD | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | ENZ |  | ITMD | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | ENZ |  | ITMD | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | ENZ |  | ITMD | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | ENZ |  | ITMD | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | ENZ |  | ITMD | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | FDB |  | WCON |  | NC | 70 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | FDB |  | FCNS |  | NC | 84 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | FDB |  | WCON |  | NC | 84 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | FDB |  | WCON |  | NC | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | FDB |  | FCNS |  | NC | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | FDB |  | WCON |  | NC | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | FDB |  | WCON |  | NC | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | FDB |  | FCNS |  | NC | 56 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | FDB |  | WCON |  | NC | 56 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEC | FDB |  | WCON | U | F | 19 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEC | FDB |  | WCON | U | F | 33 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 8 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | GRO |  | WGHT |  | NC | 56 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEC | GRO |  | WGHT | U | F | 6 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 9.6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEC | GRO |  | GAIN | U | F | 33 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 8 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEC | GRO |  | WGHT | U | F | 19 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | GRO |  | WGHT | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | GRO |  | WGHT | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | GRO |  | WGHT | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | GRO |  | WGHT | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | GRO |  | WGHT | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | HRM |  | DOPA | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | HRM |  | DOPA | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | HRM |  | THYR | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | HRM |  | TSHT | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | HRM |  | THYR | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | HRM |  | TRII | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | HRM |  | TSHT | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | HRM |  | THYR | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | HRM |  | TRII | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | HRM |  | TSHT | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEC | MPH |  | WGHT | U | F | 6 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 4.8 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEC | MPH |  | WGHT | U | F | 6 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 4.8 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEC | PHY |  | BDVL | U | F | 6 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 9.6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEC | PHY |  | FLUX | U | F | 6 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 9.6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RESP | U | F | 17 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RPRT | U | F | 17 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RESP | U | F | 17 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RESP | U | F | 17 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RESP | U | F | 26 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RESP | U | F | 26 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RPRT | U | F | 26 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RESP | U | F | 26 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RESP | U | F | 30 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RESP | U | F | 30 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RPRT | U | F | 30 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RESP | U | F | 30 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RESP | U | F | 34 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RESP | U | F | 34 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RPRT | U | F | 34 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RESP | U | F | 34 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RESP | U | F | 18 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RPRT | U | F | 18 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RESP | U | F | 18 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RESP | U | F | 18 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RESP | U | F | 22 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RESP | U | F | 22 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RPRT | U | F | 22 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RESP | U | F | 22 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | RESP | U | F | 22 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEC | PHY |  | IUPT | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEC | REP |  | PROG | U | F | 19 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEC | REP |  | PROG | U | F | 24 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEC | REP |  | PROG | U | F | 24 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | BCM |  | MTLN | U | F | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | IJ | 2 | NOEL | BCM |  | MCON | U | F | 84 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | BCM |  | PRCO | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 mg/org |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | BCM |  | PRCO | U | F | 12 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | BCM |  | GLUC | U | F | 17.5 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 25 mg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | BCM |  | GLUC | U | F | 17.5 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 25 mg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | BCM |  | PRCO | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | GV | 2 | NOEL | BCM |  | PRCO | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.04 mg/100g bw |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | BCM |  | CHOL | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | BCM |  | CHOL | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | BCM |  | CHOL | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | GV | 2 | NOEL | BCM |  | CHOL | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.3 mg/100g bw/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | NOEL | BCM |  | AION | U | F | 50 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 ug/ml |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | BCM |  | PRCO | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | BCM |  | PRCO | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | BCM |  | PRCO | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 4 | NOEL | BCM |  | PRCO | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.5 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEL | BEH |  | STPY | U | F | 33 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.5 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | NOEL | BEH |  | VMVT | U | F | 127 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | BEH |  | ACTP | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.45 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 4 | NOEL | BEH |  | ACTP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.45 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEL | BEH |  | ACTV | U | F | 19 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 12 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | BEH |  | ACTV | U | F | 3 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | DR | 3 | NOEL | BEH |  | NMVM | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 mg/L |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | NOEL | BEH |  | NMVM | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 mg/L |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | NOEL | BEH |  | NMVM | U | F | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 mg/L |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | BEH |  | ACTV | U | A | 52 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg bdwt |  |
| Rattus norvegicus | | NONE | LAB | DM | 2 | NOEL | BEH |  | ACTV | U | F | 44 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 5 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 4 | NOEL | BEH |  | RRSP | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | IP | 5 | NOEL | BEH |  | GBHV | U | F | 13 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.60 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | NOEL | CEL |  | RSBC | U | NC | 119 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 4 | NOEL | CEL |  | NCEL | U | A | 4.083 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.5 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | IP | 2 | NOEL | CEL |  | NCEL | U | F | 6 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | CEL |  | RSBC | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | IM | 3 | NOEL | CEL |  | DNSY | U | F | 32 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 25 mg |  |
| Rattus norvegicus | | CUL | LAB | CM | 4 | NOEL | DVP |  | DFRM | U | F | 2 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 100 uM |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 150 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 150 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 150 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | DVP |  | GDVP | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 150 ug/h |  |
| Rattus norvegicus | | NONE | LAB | IJ | 2 | NOEL | ENZ |  | CRLP | U | F | 84 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | GV | 2 | NOEL | ENZ |  | ALPH | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.04 mg/100g bw |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | ENZ |  | CACA | U | A | 10 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.6 uM/kg |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | ENZ |  | MALE | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | ENZ |  | MALE | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | ENZ |  | MALE | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | ENZ |  | TSHX | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | ENZ |  | TSHX | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | ENZ |  | TSHX | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | ENZ |  | TSHX | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | DR | 4 | NOEL | FDB |  | WCON | M | A | 50 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 5.6 ug/ml |  |
| Rattus norvegicus | | NONE | LAB | SC | 4 | NOEL | FDB |  | FCNS | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.6 mg/kg bdwt |  |
| Rattus norvegicus | | NONE | LAB | SC | 4 | NOEL | FDB |  | FCNS | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg bdwt |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | GEN |  | DNAC | U | F | 84 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 mg/org |  |
| Rattus norvegicus | | NONE | LAB | GV | 2 | NOEL | GEN |  | DNAC | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.04 mg/100g bw |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | GEN |  | MRNA | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | GEN |  | MRNA | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | GEN |  | MRNA | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | GEN |  | MRNA | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | GRO |  | WGHT | U | A | 100 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.0 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | LC | 2 | NOEL | GRO |  | WGHT | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1 mg/kg bdwt |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | GRO |  | WGHT | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 mg/org |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | GRO |  | WGHT | U | F | 84 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 mg/org |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | NOEL | GRO |  | WGHT | U | F | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | GRO |  | WGHT | U | F | 12 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEL | GRO |  | WGHT | U | F | 19 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 8 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | GRO |  | WGHT | U | F | 61 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | GRO |  | WGHT | U | F | 61 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | GRO |  | WGHT | U | F | 17.5 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 25 mg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | GRO |  | WGHT | U | F | 17.5 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 25 mg |  |
| Rattus norvegicus | | NONE | LAB | MU | 3 | NOEL | GRO |  | WGHT | U | F | 75 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 4.5 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | LC | 3 | NOEL | GRO |  | WGHT | U | F | 75 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 4.5 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | GRO |  | WGHT | U | A | 107 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.4 mg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 3 | NOEL | GRO |  | WGHT | U | F | 107 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 5.6 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 4 | NOEL | GRO |  | GAIN | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.6 mg/kg bdwt |  |
| Rattus norvegicus | | NONE | LAB | SC | 4 | NOEL | GRO |  | GAIN | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg bdwt |  |
| Rattus norvegicus | | NONE | LAB | DR | 3 | NOEL | GRO |  | WGHT | M | A | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.391 mg/100g bw |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | GRO |  | WGHT | U | F | 35 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg |  |
| Rattus norvegicus | | CUL | LAB | CM | 4 | NOEL | GRO |  | DMTR | U | F | 2 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 100 uM |  |
| Rattus norvegicus | | NONE | LAB | IM | 3 | NOEL | GRO |  | WGHT | U | F | 32 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 25 mg |  |
| Rattus norvegicus | | NONE | LAB | DM | 2 | NOEL | GRO |  | WGHT | U | F | 5 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 5.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 4 | NOEL | GRO |  | GAIN | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.5 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | NOEL | GRO |  | WGHT | U | F | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.77 mM |  |
| Rattus norvegicus | | NONE | LAB | GV | 2 | NOEL | GRO |  | GAIN | U | F | 53 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | GV | 2 | NOEL | GRO |  | GAIN | U | F | 53 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | HIS |  | LESI | U | F | 4 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 8 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 3 | NOEL | HIS |  | LESI | U | F | 4 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 8 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 5 | NOEL | HIS |  | NCRO | U | F | 35 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | NOEL | HRM |  | CORT | U | F | 127 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | HRM |  | CORT | U | F | 11 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | HRM |  | DOPA | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.8 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | HRM |  | DOPA | U | F | 8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.8 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | IA | 2 | NOEL | HRM |  | EPIN | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.1 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | HRM |  | INSL | U | F | 17.5 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 25 mg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | HRM |  | INSL | U | F | 17.5 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 25 mg |  |
| Rattus norvegicus | | NONE | LAB | MU | 3 | NOEL | HRM |  | LUTH | U | F | 75 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2.4 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | MU | 3 | NOEL | HRM |  | LUTH | U | F | 75 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2.4 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | NOEL | HRM |  | LUTH | U | F | 75 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 4.5 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | LC | 3 | NOEL | HRM |  | LUTH | U | F | 75 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 4.5 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | LC | 3 | NOEL | HRM |  | LUTH | U | F | 75 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2.4 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | NOEL | HRM |  | NORE | U | NC | 77 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | THYR | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | DOPA | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 3 | NOEL | HRM |  | DOPA | U | F | 77 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | NOEL | HRM |  | INSL | U | F | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.77 mM |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | NOEL | HRM |  | INSL | U | F | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.77 mM |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | HRM |  | VASO | U | F | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | HRM |  | VASO | U | F | 29 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | HRM |  | VASO | U | F | 29 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | HRM |  | VASO | U | F | 35 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | HRM |  | VASO | U | F | 35 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | HRM |  | VASO | U | F | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | HRM |  | VASO | U | F | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | HRM |  | VASO | U | F | 56 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | HRM |  | VASO | U | F | 56 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | HRM |  | VASO | U | F | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 19 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 19 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 19 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 19 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 26 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 26 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 26 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 26 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 33 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 33 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 33 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 33 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 33 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 47 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 47 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 47 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 47 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 19 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 26 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 47 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 47 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 47 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 47 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 47 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 61 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 61 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 61 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 40 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 47 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 47 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 60 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | HRM |  | VASO | U | F | 61 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | NOEL | INJ |  | DAMG | U | F | 50 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 ug/ml |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEL | MOR |  | SURV | U | F | 24 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 3 | NOEL | MOR |  | LFSP | U | F | 62.8 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 mg/L |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | NOEL | MOR |  | LFSP | U | F | 27.5 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 mg/L |  |
| Rattus norvegicus | | NONE | LAB | IM | 2 | NOEL | MOR |  | LFSP | U | F | 571.4 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.42 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | MPH |  | SMIX | U | A | 100 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1.0 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | MPH |  | WGHT | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 mg/org |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | MPH |  | WGHT | U | F | 84 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 50 mg/org |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | NOEL | MPH |  | WGHT | U | F | 127 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | MPH |  | WGHT | U | F | 12 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.4 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | DT | 4 | NOEL | MPH |  | DMTR | U | F | 9 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 5 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | NOEL | MPH |  | WGHT | U | F | 4 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.03 mg/ml |  |
| Rattus norvegicus | | NONE | LAB | IP | 2 | NOEL | MPH |  | WGHT | U | F | 30 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEL | MPH |  | WGHT | U | F | 6 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 9.6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | GV | 2 | NOEL | MPH |  | WGHT | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.3 mg/100g bw/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 3 | NOEL | MPH |  | WGHT | M | A | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.391 mg/100g bw |  |
| Rattus norvegicus | | NONE | LAB | GV | 3 | NOEL | MPH |  | VOLU | U | F | 30 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 7.5 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | MPH |  | WGHT | U | F | 35 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2 mg/kg |  |
| Rattus norvegicus | | CUL | LAB | CM | 4 | NOEL | MPH |  | SMTE | U | F | 2 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 100 uM |  |
| Rattus norvegicus | | NONE | LAB | IM | 3 | NOEL | MPH |  | WGHT | U | F | 32 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 25 mg |  |
| Rattus norvegicus | | NONE | LAB | DT | 4 | NOEL | MPH |  | WGHT | U | F | 58 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.06% |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | MPH |  | SMTE | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 | NOEL | MPH |  | SMTE | U | F | 7 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | PHY |  | SYPS | U | F | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | IJ | 2 | NOEL | PHY |  | SYPS | U | F | 84 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | IJ | 2 | NOEL | PHY |  | SYPS | U | F | 84 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 75 ug/h |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | PHY |  | SYPS | U | F | 17.5 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 25 mg |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NOEL | PHY |  | SYPS | U | F | 17.5 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 25 mg |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | NOEL | PHY |  | SYPS | U | F | 35 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 100 mg/L |  |
| Rattus norvegicus | | NONE | LAB | IJ | 2 | NOEL | PHY |  | OXYG | U | A | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.4 mg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | PHY |  | BLPR | U | F | 17.5 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 2.4 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 4 | NOEL | PHY |  | BTMP | U | F | 21 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 | NOEL | REP |  | GSTT | U | F | 24 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | NOEL | REP |  | PROG | U | F | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 | NOEL | REP |  | PROG | U | F | 19 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | MU | 3 | NOEL | REP |  | VAOP | U | F | 75 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 4.5 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | LC | 3 | NOEL | REP |  | VAOP | U | F | 75 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 4.5 mg/kg bdwt/d |  |
| Rattus norvegicus | | NONE | LAB | IP | 2 | NOEL | REP |  | SPMC | U | F | 30 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 1 mg/kg |  |
| Rattus norvegicus | | NONE | LAB | GV | 2 | NOEL | REP |  | SPCL | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.04 mg/100g bw |  |
| Rattus norvegicus | |  | LAB | DT | 2 | NOEL | REP |  | DSTR | U | F | 15 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.3 mg/100g bw/d |  |
| Rattus norvegicus | | NONE | LAB | GV | 2 | NOEL | REP |  | SPCL | U | F | 31 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.3 mg/100g bw/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 |  | ACC |  | RSDE | U | F | 6 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | (2.4-9.6) mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 |  | ACC |  | RSDE | U | F | 14 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 0.1 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 |  | BEH |  | ACTV | U | F | 33 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | (0.75-3) mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 |  | BEH |  | ACTV | U | F | 33 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | (0.75-3) mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 |  | BEH |  | ACTV | U | F | 33 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | (0.75-3) mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 |  | BEH |  | ACTV | U | F | 33 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | (0.75-3) mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 |  | BEH |  | ACTV | U | F | 33 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | (0.75-3) mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 |  | FDB |  | WCON | U | F | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | (0.6-6) mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 |  | FDB |  | FCNS | U | F | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | (0.6-6) mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 4 |  | GRO |  | GAIN | U | F | 6 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | (2.4-9.6) mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 |  | GRO |  | WGHT | U | F | 17-34 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 |  | GRO |  | GAIN | U | F | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | (0.6-6) mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 |  | HRM |  | VASO | U | F | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | (0.6-6) mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 |  | HRM |  | VASO | U | F | 19-61 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 |  | HRM |  | VASO | U | F | 19-61 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 |  | HRM |  | VASO | U | F | 19-61 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 |  | HRM |  | VASO | U | F | 19-61 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 2 |  | HRM |  | VASO | U | F | 19-61 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 |  | MPH |  | WGHT | U | F | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | (0.6-6) mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | OP | 3 |  | PHY |  | OSMO | U | F | 28 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | (0.6-6) mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | DR | 2 | NR-ZERO | MOR |  | MORT | U | NC | 42 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 6.0 mg/kg/d |  |
| Rattus norvegicus | | NONE | LAB | SC | 2 | NR-ZERO | MOR |  | MORT |  | A | 547.92 d |
| Norway Rat | |  |  |  |  |  |  |  |  |  | 3.0 mg/kg bdwt |  |

As taken from the US EPA ECOTOX database

## *5.3.* *Reproduction toxicity*

“Nicotine readily gains access to the fetal compartment via the placenta, with fetal concentration generally higher than maternal levels (Koren G, 1995). Nicotine concentrations in amniotic fluid sampled during mid-trimester amniocentesis were found to be 54% higher than the corresponding maternal serum level. Placental tissue, from first trimester abortions and term deliveries, was also noted to concentrate nicotine (Luck W et al., 1985). Nicotine also passes freely into breastmilk with a milk-to-maternal plasma ration of 2.9 (Luck W & Nau H, 1984). The physiological effect of nicotine on fetal growth seems to be vasoconstriction of the uterine and the umbilical artery.” (Lambers and Clark 1996).

The safety of NRT use during pregnancy has been evaluated in a limited number of short-term human trials, but there is currently no information on the long-term effects of developmental nicotine exposure in humans.

Schroeder et al., (2002) evaluated the delivery outcomes in pregnant patients (n=21) who smoked ≥ 15 cigarettes per day during the third and who utilized 22 mg/day nicotine patch systems for 8 weeks in a prospective, open-label trial. Birth outcomes included a mean weight of 3405 ± 570 g, gestation age of 39 ± 1.3 weeks, 1-minute Apgar score of 7.7 ± 2.1 and 5-minute Apgar score of 8.9 ± 1.9. Three infants had major malformations or complications, including complete transposition of the great vessels (develops prior to 30 weeks’ gestation, therefore was not considered as a result of NRT), fetal asystole and mild respiratory distress and non-epileptic seizures less than 1 month after delivery, then progressed normally. Despite its limitations, this trail indicated that birth outcomes of patch users appear to be similar to the general population birth weight of 3389 ± 466 g at 37-40 weeks of gestation.

In another, intention-to treat trial, women who smoked ≥ 10 cigarettes per day and were less than 22 weeks pregnant, received behavioral modification therapy and were randomly assigned either to a 15-mg NRT patch for 8 weeks followed by a 10-mg patch for 3 weeks (n=120), or to placebo (n=122). No significant difference was observed between NRT and placebo groups for overall birth weight (3457 vs. 3271 g, respectively). Birth weight for those born after 37 weeks of gestation (3539 vs. 3381 g, respectively), birth weight less than 2500 g (3% vs. 9%, respectively), or preterm delivery (8% vs. 10%, respectively). Although this was an intent-to treat trial, one limitation was that only 11% of patients completed the full course therapy. Other limitations include use of the 16-hour formulation, a low cessation rate and use of the same strength parch regardless of the baseline number of cigarettes smoked (Wisborg et al. 2000).

Oncken et al., (2008) analyzed the effects NRT gum on pregnancy. A randomized, double-blinded, controlled trial compared the use of 2-mg gum (n=100) versus placebo (n=94) for 6 weeks in women less than 26 weeks pregnant and who smoked at least 1 cigarette per day. The NRT group had a higher birth weight (3287 vs. 2950), decreased risk of low birth weight, defined as less than 2500 g (2% vs. 18%) and lower risk of preterm delivery, defined as before 37 weeks of gestation. (7.2% vs. 18%). No significant differences existed in Apgar scores at 1 and 5 min, birth length, head circumference or length of neonatal hospital stay. This study had a few limitations including usage of the NRT gum was not revealed, there was continued smoking while the gum was used and the study did not achieved 80% of power.

“A retrospective cohort study examined the malformation rate in Danish women who smoked (n=16812) or used the patch, gum or nasal spray NRT (n=250) during their first trimester compared to nonsmokers (n=55915). NRT users had an increased risk for any malformation but not for major or musculoskeletal malformations. Beside the hip dislocations, specific types of abnormalities were not reported or compared to an expected rate within the general population. Other limitations include recall bias in smoking rate during the first trimester, the small number of NRT used and non-provision of type, dose or duration of NRT used, and non-evaluation of concomitant medication use. Overall, the NRT use in the first trimester increased the risk for all malformations but not major, however, the study’s many limitations decrease the generalizability of these data and further studies are needed.” (Morales-Suarez-Varela et al. 2006).

Effects on development

“Clinical studies showed that intrauterine growth retardation (IUGR) neonatus had lower cholesterol concentrations in cord blood, which might be associated with increased risk of metabolic syndrome and cardiovascular diseases in adulthood. We previously observed lower blood cholesterol levels in prenatal nicotine exposure (PNE)-induced IUGR fetal rats, and this study aimed to elucidate the placental mechanism. Pregnant Wistar rats were subcutaneously injected with nicotine (2.0 mg/kg⋅d) on gestational day 9-20. In vivo, PNE increased levels of total cholesterol (TCH), high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) in maternal serum, while decreased levels of TCH and LDL-C in female fetal serum. Meanwhile, the expression of scavenger receptor class B type 1 (SR-B1), ATP-binding cassette transporter A1 (ABCA1) and ATP-binding cassette transporter G1 (ABCG1) were decreased, and the expression of liver X receptor (LXR) α and β were also decreased in female placentas. In vitro, nicotine (0.1-10 μM) reduced the expression of LXRα, LXRβ, SR-B1, ABCA1 and ABCG1 in a concentration dependent manner, which could be annulled by nAChR antagonist and LXR agonist. Taken together, nicotine could inhibit the expression of SR-B1, ABCA1 and ABCG1 via nAChR and LXR α/β in female placentas, finally leading to reduced blood cholesterol levels in fetal rats.” (Zhan G et al. 2018).

“E-cigarette usage is increasing, especially among the young, with both the general population and physicians perceiving them as a safe alternative to tobacco smoking. Worryingly, e-cigarettes are commonly used by pregnant women. As nicotine is known to adversely affect children in utero, we hypothesized that nicotine delivered via e-cigarettes would negatively affect lung development. To test this, we developed a mouse model of maternal e-vapor (nicotine and nicotine-free) exposure and investigated the impact on the growth and lung inflammation in both offspring and mothers. Female Balb/c mice were exposed to e-fluid vapor containing nicotine (18 mg/ml nicotine E-cigarette [E-cig18], equivalent to two cigarettes per treatment, twice daily,) or nicotine free (E-cig0 mg/ml) from 6 weeks before mating until pups weaned. Male offspring were studied at Postnatal Day (P) 1, P20, and at 13 weeks. The mothers were studied when the pups weaned. In the mothers' lungs, e-cigarette exposure with and without nicotine increased the proinflammatory cytokines IL-1β, IL-6, and TNF-α. In adult offspring, TNF-α protein levels were increased in both E-cig18 and E-cig0 groups, whereas IL-1β was suppressed. This was accompanied by global changes in DNA methylation. In this study, we found that e-cigarette exposure during pregnancy adversely affected maternal and offspring lung health. As this occurred with both nicotine-free and nicotine-containing e-vapor, the effects are likely due to by-products of vaporization rather than nicotine.” (Chen H et al. 2018).

“Maternal nicotine (NIC) exposure causes overweight, hyperleptinemia and metabolic disorders in adult offspring. Our study aims to explore the underlying mechanism of perinatal NIC exposure increases obesity susceptibility in adult female rat offspring. In our model, we found that adult NIC-exposed females presented higher body weight and subcutaneous and visceral fat mass, as well as larger adipocytes, while no change was found in food intake. Serum profile showed a higher serum glucose, insulin and leptin levels in NIC-exposed females. In adipose tissue and liver, the leptin signaling pathway was blocked at 26 weeks, presented lower Janus tyrosine kinase 2 and signal transducer and activator of transcription 3 gene expression, higher suppressor of cytokine signaling 3 gene expression (in adipose tissue) and lower leptin receptors gene expression (in liver), indicating that peripheral leptin resistance occurred in NIC-exposed adult females. In female rats, the expression of lipolysis genes was affected dominantly in adipose tissue, but lipogenesis genes was affected in liver. Furthermore, the glucose and insulin tolerance tests showed a delayed glucose clearance and a higher area under the curve in NIC-exposed females. Therefore, perinatal NIC exposure programed female rats for adipocyte hypertrophy and obesity in adult life, through the leptin resistance in peripheral tissue.” (Zhang W et al. 2018).

“METHODS: Pregnant C57 mice drank nicotine- or cotinine-laced water for 6 wks from conception (NPRE = 2% saccharin + 100 μg nicotine/mL; CPRE = 2% saccharin + 10 μg cotinine/mL) or 3 wks after birth (CPOST = 2% saccharin + 30 μg cotinine/mL). Controls drank 2% saccharin (CTRL). At 17 ± 1 weeks (male pups; CTRL n = 6; CPOST n = 6; CPRE n = 8; NPRE n = 9), we assessed (i) cardiovascular control during sleep; (ii) arterial reactivity ex vivo; and (iii) expression of genes involved in arterial constriction/dilation. RESULTS: Blood cotinine levels recapitulated those of passive smoker mothers' infants. Pups exposed to cotinine exhibited (i) mild bradycardia - hypotension at rest (p < 0.001); (ii) attenuated (CPRE , p < 0.0001) or reverse (CPOST ; p < 0.0001) BP stress reactivity; (iii) adrenergic hypocontractility (p < 0.0003), low protein kinase C (p < 0.001) and elevated adrenergic receptor mRNA (p < 0.05; all drug-treated arteries); and (iv) endothelial dysfunction (NPRE only). CONCLUSION: Cotinine has subtle, enduring developmental consequences. Some cardiovascular effects of nicotine can plausibly arise via conversion into cotinine. Low-level exposure to this metabolite may pose unrecognised perinatal risks. Adults must avoid inadvertently exposing a foetus or infant to cotinine as well as nicotine.” (Bastianini et al. 2018).

“The adverse effects of prenatal nicotine and alcohol exposure on human reproductive outcomes are a major scientific and public health concern. In the United States, substantial percentage of women (20-25%) of childbearing age currently smoke cigarettes and consume alcohol, and only a small percentage of these individuals quit after learning of their pregnancy. However, there are very few scientific reports on the effect of nicotine in prenatal alcohol exposure on the cerebellum of the offspring. Therefore, this study was conducted to investigate the cerebellar neurotoxic effects of nicotine in a rodent model of Fetal Alcohol Spectrum Disorder (FASD). In this study, we evaluated the behavioral changes, biochemical markers of oxidative stress and apoptosis, mitochondrial functions and the molecular mechanisms associated with nicotine in prenatal alcohol exposure on the cerebellum. Prenatal nicotine and alcohol exposure induced oxidative stress, did not affect the mitochondrial functions, increased the monoamine oxidase activity, increased caspase expression and decreased ILK, PSD-95 and GLUR1 expression without affecting the GSK-3β. Thus, our current study of prenatal alcohol and nicotine exposure on cerebellar neurotoxicity may lead to new scientific perceptions and novel and suitable therapeutic actions in the future.” (Bhattacharya et al 2018).

“This study investigates the transfer of nicotine from lactating dams to their offspring through breast milk, in the frame of a research focused to ascertain toxicological and neuro-behavioural effects on pups as consequence of either unavoidable ("yoked & forced") or voluntary ("freely-chosen") maternal nicotine exposure. To this aim, plasmatic concentrations of nicotine and cotinine were determined by LC-MS/MS in Wistar rat pups whose mothers were orally administered with nicotine during lactation. Mothers were divided into a voluntary drinking group, an unavoidable consumption group, and controls. The limits of detection and quantification of the LC-MS/MS method were 0.20 and 0.65 ng/mL, respectively. Within-laboratory reproducibility (CV%) was <12%, with recovery of 86.2-118.8%. Results showed the presence of nicotine in 67% of samples from freely-chosen consumption group (1.30 ± 0.31 ng/mL) and in 60% of samples from yoked-consumption group (1.19 ± 0.62 ng/mL); cotinine was found in all the samples from freely-chosen (1.92 ± 0.77 ng/mL) and yoked-consumption groups (1.43 ± 0.30 ng/mL). Data provide an evidence-based support to maternal/offspring nicotine transfer as function of different ways of oral exposure.” (Famele,et al. 2018).

“Prenatal nicotine exposure (PNE) is closely related to depression in offspring. However, the underlying mechanism is still unclear. We hypothesized that neurosteroid in the hippocampus may mediate PNE‑induced depression‑like behaviors. Nicotine was subcutaneously administered (1.0 mg/kg) to pregnant rats twice daily from gestational day (GD) 9 to 20. In adolescent offspring, PNE significantly increased immobility time and decreased the sucrose preference in female rats. The numbers of hippocampal neurons declined in the CA3 and DG regions. Steroidogenic acute regulatory protein (StAR) expression was suppressed in female rats. In fetal offspring, the neuronal numbers of CA3 regions in PNE female fetal hippocampal were significantly decreased, accompanied by the enhanced content of corticosterone and StAR expression. These data indicated that PNE induced depression‑like behavior in adolescent female rats via the regulation of neurosteroid levels in the hippocampus.” (Zhang C et al. 2019).

“Prenatal nicotine exposure (PNE) could induce ovarian dysplasia in offspring. This study aimed to confirm its intrauterine origin and explore a programming mechanism of ovarian dysplasia caused by PNE. Pregnant Wistar rats were injected subcutaneously with nicotine (2 mg/kg.d) from gestation day (GD) 9 to GD20. Serum of female offspring was obtained for hormone assays and ovarian tissues were collected. The results showed that PNE impaired ovarian development, and inhibited estradiol production and cytochrome P450 aromatase (P450arom) expression before and after birth. Moreover, the nicotinic acetylcholine receptors (nAChRs) expression was increased in utero, while histone 3 lysine 9 acetylation (H3K9ac) and H3K27ac levels in the P450arom promoter region were decreased persistently in PNE group before and after birth. In vitro, nicotine decreased P450arom expression and estradiol production in human granulosa cell line KGN. Furthermore, nicotine treatment up-regulated nAChRα6 and α9 expression and down-regulated the H3K9ac and H3K27ac levels of the P450arom promoter region. Non-specific nAChRs inhibitor vecuronium bromide reversed these effects. These results suggest that PNE could induce ovarian dysplasia and inhibit estradiol synthesis in the female offspring rats, which was related to the decreased H3K9ac and H3K27ac levels in the promotor region of the P450arom via the nAChRs.” (Fan et al. 2019).

“Nicotine has been recognized to trigger various neuronal disabilities in the fetal brain and long-lasting behavioral deficits in offspring. However, further understanding of fetal brain development under nicotine exposure is challenging due to the limitations of existing animal models. Here, we create a new brain organoid-on-a-chip system derived from human induced pluripotent stem cells (hiPSCs) that allows us to model neurodevelopmental disorders under prenatal nicotine exposure (PNE) at early stages. The brain organoid-on-a-chip system facilitates 3D culture, in situ neural differentiation, and self-organization of brain organoids under continuous perfused cultures in a controlled manner. The generated brain organoids displayed well-defined neural differentiation, regionalization, and cortical organization, which recapitulates the key features of the early stages of human brain development. The brain organoids exposed to nicotine exhibited premature neuronal differentiation with enhanced expression of the neuron marker TUJ1. Brain regionalization and cortical development were disrupted in the nicotine-treated organoids identified by the expressions of forebrain (PAX6 and FOXG1), hindbrain (PAX2 and KROX20) and cortical neural layer (preplate TBR1 and deep-layer CTIP2) markers. Moreover, the neurite outgrowth showed abnormal neuronal differentiation and migration in nicotine-treated brain organoids. These results suggest that nicotine exposure elicits impaired neurogenesis in early fetal brain development during gestation. The established brain organoid-on-a-chip system provides a promising platform to model neurodevelopmental disorders under environmental exposure, which can be extended for applications in brain disease studies and drug testing.” (Wang Y et al. 2018b).

“Impaired placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) activity which inactivates maternal glucocorticoids is associated with poor fetal growth and a higher risk of chronic diseases in adulthood. This study aimed to elucidate the epigenetically regulatory mechanism of nicotine on placental 11β-HSD2 expression. Pregnant Wistar rats were administered 1.0 mg/kg nicotine subcutaneously twice a day from gestational day 9 to 20. The results showed that prenatal nicotine exposure increased corticosterone levels in the placenta and fetal serum, disrupted placental morphology and endocrine function, and reduced fetal bodyweight. Meanwhile, histone modification abnormalities (decreased acetylation and increased di-methylation of histone 3 Lysine 9) on the HSD11B2 promoter and lower-expression of 11β-HSD2 were observed. Furthermore, the expression of nicotinic acetylcholine receptor (nAChR) α4/β2, the phosphorylation of extracellular regulated kinase 1/2 (ERK1/2) and Ets-like protein-1 (Elk-1), and the expression of early growth response-1 (Egr-1) were increased in the nicotine groups. In human BeWo cells, nicotine decreased 11β-HSD2 expression, increased nAChRα9 expression, and activated ERK1/2/Elk-1/Egr-1 signaling in the concentration (0.1-10 μM)-dependent manner. Antagonism of nAChRs, inhibition of ERK1/2 and Egr-1 knockdown by siRNA were able to block/abrogate the effects of nicotine on histone modification and expression of 11β-HSD2. Taken together, nicotine can impair placental structure and function, and induce fetal developmental toxicity. The underlying mechanism involves histone modifications and down-regulation of 11β-HSD2 through nAChRs/ERK1/2/Elk-1/Egr-1 signaling, which increases active glucocorticoids levels in the placenta and fetus, and eventually inhibits the fetal development.” (Zhou J et al. 2018).

“Maternal smoking has negative long-term consequences on affective behaviors, and in rodents, chronic neonatal nicotine exposure (CNN) results in increased anxiety. In rat pups, acute nicotine stimulation activates brain regions associated with stress and anxiety, but chronic nicotine exposure could desensitize of nicotinic acetylcholine receptors, the molecular target of nicotine. Here, we determined whether CNN affected neuronal activation by an acute nicotine challenge. Using in situ hybridization, we analyzed mRNA expression of the immediate-early genes (IEGs) c-Fos, Arc, Egr-1 and Npas4, which are markers for neuronal activation and implicated in synaptic plasticity. Following CNN (6 mg/kg/day) or control treatment from postnatal day (P)1 to P7, an acute i.p. nicotine (0.7 mg/kg) or saline injection (control) was administered on P8, and brains collected after 30 min. In drug-naive pups, acute nicotine stimulated IEGs expression specifically in brain areas associated with innate anxiety including the paraventricular hypothalamic nucleus, central nucleus of the amygdala (CeA), and locus coeruleus (LC). Following CNN, acute nicotine stimulated IEG expression in all three areas, but activation was significantly reduced in the LC (c-Fos, Egr-1, Npas4), and CeA (c-Fos). Notably, nicotine-induced Npas4 expression was greatly diminished in the LC, which may affect inhibitory synapse formation in noradrenergic neurons. Thus, after CNN, neurons located in areas associated with anxiety brain circuitry maintained responsiveness to nicotine, but tolerance differentially developed to nicotine. In the developing brain, repeated activation by nicotine of areas related to limbic pathways could alter circuit connectivity and increase responsiveness to stress and anxiety later in life.” (Halawa et al. 2018).

“Globally, approximately 10%-25% of women smoke during pregnancy. Since nicotine is highly addictive, women may use nicotine-containing products like nicotine replacement therapies for smoking cessation, but the long-term consequences of early life exposure to nicotine remain poorly defined. Our laboratory has previously demonstrated that maternal nicotine exposed (MNE) rat offspring exhibit hypertriglyceridemia due to increased hepatic de novo lipogenesis. Hypertriglyceridemia may also be attributed to impaired white adipose tissue (WAT) lipid storage; however, the effects of MNE on WAT are not completely understood. We hypothesize that nicotine-induced alterations in adipose function (eg, lipid storage) underlie dyslipidemia in MNE adults. Female 6-month-old rats exposed to nicotine during gestation and lactation exhibited significantly decreased visceral adipocyte cell area by 40%, attributed, in part, to a 3-fold increase in adipose triglyceride lipase (ATGL) protein expression compared with vehicle. Given ATGL has antioxidant properties and in utero nicotine exposure promotes oxidative stress in various tissues, we next investigated if there was evidence of increased oxidative stress in MNE WAT. At both 3 weeks and 6 months, MNE offspring expressed 37%-48% higher protein levels of superoxide dismutase-1 and -2 in WAT. Since oxidative stress can induce inflammation, we examined the inflammatory profile of WAT and found increased expression of cytokines (interleukin-1β, tumor necrosis factor α, and interleukin-6) by 44%-61% at 6 months. Collectively, this suggests that the expression of WAT ATGL may be induced to counter MNE-induced oxidative stress and inflammation. However, higher levels of ATGL would further promote lipolysis in WAT, culminating in impaired lipid storage and long-term dyslipidemia.” (Barra et al. 2018).

“Accumulating evidence has shown that the impact of prenatal environmental factors on the organs of the offspring could last until the adulthood. Here, we aimed to investigate these effects and the potential mechanism of prenatal nicotine exposure (PNE) on the female adult cartilage of the first generation (PNE-F1) and the second generation (PNE-F2). Pregnant Wistar rats were injected with 2.0 mg/kg.d nicotine from gestational day (GD) 9 to 20. Then their F1 generation at GD20 and postnatal week (PW) 12, and F2 generation at PW12 were harvested. The expression of extracellular matrix (ECM) and transforming growth factor β (TGFβ) signaling genes were analyzed by real-time quantitative PCR, and the histone acetylation was examined by chromatin immunoprecipitation assay. The results showed that PNE reduced the ECM and TGFβ signaling gene expressions in both PNE-F1 and PNE-F2 female adult articular cartilage. In the F1 generation, PNE inhibited the acetylation at H3K9 of TGFβ, TGFβ receptor 1 (TGFβR1), SRY-type high mobility group box 9 (SOX9), a1 chain of type II collagen (COL2A1) and aggrecan (ACAN) gene promoters at both GD20 and PW12. In PNE-F2 at PW12, the obvious deacetylation at H3K9 of the TGFβR1 and COL2A1 promoters still existed. Moreover, in rat fetal chondrocytes, corticosterone rather than nicotine directly induced the hypoacetylation of H3K9 of TGFβR1 and COL2A1 genes, which might be the main cause of imperfect cartilage for PNE-F2. This study may be helpful to elucidate the developmental variability of articular cartilage quality and useful for the early prevention of articular damage.” (Xie et al. 2018).

“Despite dissemination of information regarding the harm on fetal development of smoking while pregnant, the number of pregnancies associated with nicotine exposure appears to have stagnated. Presence of nicotine during neural formulation is associated with a higher susceptibility of drug dependence, suggesting an altered development of neurons in circuits involved in saliency and motivation. The laterodorsal tegmental nucleus (LDT) plays a role in coding stimuli valence via afferents to mesolimbic nuclei. Accordingly, alterations in development of neural mechanisms in the LDT could be involved in vulnerability to drug dependency. Therefore, we examined the effect of prenatal nicotine exposure (PNE) on glutamatergic functioning of LDT neurons in mouse brain slices using whole-cell, patch clamp concurrent with fluorescence-based calcium imaging. PNE was associated with larger amplitudes of AMPA-induced currents, and greater AMPA-mediated rises in intracellular calcium. AMPA/NMDA ratios and the AMPA-current rectification index were lower and higher, respectively, consistent with changes in the functionality of AMPA receptors in the PNE, which was substantiated by a greater inhibition of evoked and spontaneous glutamatergic synaptic events by a selective inhibitor of GluA2-lacking AMPA receptors. Paired pulse ratios showed a decreased probability of glutamate release from presynaptic inputs, and fluorescent imaging indicated a decreased action potential-dependent calcium increase associated with PNE. When taken together, our data suggest that PNE alters LDT glutamatergic functioning, which could alter output to mesolimbic targets. Such an alteration could play a role in altered coding of relevancy of drug stimuli that could enhance risk for development of drug dependency.” (Polli and Kohlmeier 2018).

“BACKGROUND: Cigarette smoking by pregnant women is associated with a significant increase in the risk for cognitive disorders in their children. Preclinical models confirm this risk by showing that exposure of the developing brain to nicotine produces adverse behavioral outcomes. Here we describe behavioral phenotypes resulting from perinatal nicotine exposure in a mouse model, and discuss our findings in the context of findings from previously published studies using preclinical models of developmental nicotine exposure. METHODOLOGY/PRINCIPAL FINDINGS: Female C57Bl/6 mice received drinking water containing nicotine (100μg/ml) + saccharin (2%) starting 3 weeks prior to breeding and continuing throughout pregnancy, and until 3 weeks postpartum. Over the same period, female mice in two control groups received drinking water containing saccharin (2%) or plain drinking water. Offspring from each group were weaned at 3-weeks of age and subjected to behavioral analyses at 3 months of age. We examined spontaneous locomotor activity, anxiety-like behavior, spatial working memory, object based attention, recognition memory and impulsive-like behavior. We found significant deficits in attention and working memory only in male mice, and no significant changes in the other behavioral phenotypes in male or female mice. Exposure to saccharin alone did not produce significant changes in either sex. CONCLUSION/SIGNIFICANCE: The perinatal nicotine exposure produced significant deficits in attention and working memory in a sex-dependent manner in that the male but not female offspring displayed these behaviors. These behavioral phenotypes are associated with attention deficit hyperactivity disorder (ADHD) and have been reported in other studies that used pre- or perinatal nicotine exposure. Therefore, we suggest that preclinical models of developmental nicotine exposure could be useful tools for modeling ADHD and related disorders.” (Zhang L et al. 2018).

“BACKGROUND: Pregnant women may be exposed to nicotine if they smoke or use tobacco products, nicotine replacement therapy, or via e-cigarettes. Prenatal nicotine exposure has been shown to have deleterious effects on the nervous system in mammals including changes in brain size and in the dopaminergic system. The genetic and molecular mechanisms for these changes are not well understood. A Drosophila melanogaster model for these effects of nicotine exposure could contribute to faster identification of genes and molecular pathways underlying these effects. The purpose of this study was to determine if developmental nicotine exposure affects the nervous system of Drosophila melanogaster, focusing on changes to brain size and the dopaminergic system at two developmental stages. RESULTS: We reared flies on control or nicotine food from egg to 3rd instar larvae or from egg to adult and determined effectiveness of the nicotine treatment. We used immunohistochemistry to visualize the whole brain and dopaminergic neurons, using tyrosine hydroxylase as the marker. We measured brain area, tyrosine hydroxylase fluorescence, and counted the number of dopaminergic neurons in brain clusters. We detected an increase in larval brain hemisphere area, a decrease in tyrosine hydroxylase fluorescence in adult central brains, and a decrease in the number of neurons in the PPM3 adult dopaminergic cluster. We tested involvement of Dα7, one of the nicotinic acetylcholine receptor subunits, and found it was involved in eclosion, as previously described, but not involved in brain size. CONCLUSIONS: We conclude that developmental nicotine exposure in Drosophila melanogaster affects brain size and the dopaminergic system. Prenatal nicotine exposure in mammals has also been shown to have effects on brain size and in the dopaminergic system. This study further establishes Drosophila melanogaster as model organism to study the effects of developmental nicotine exposure. The genetic and molecular tools available for Drosophila research will allow elucidation of the mechanisms underlying the effects of nicotine exposure during development.” (Morris et al. 2018).

“This study investigated the mechanisms underlying the retarded development of long bone in fetus by prenatal nicotine exposure (PNE) which had been demonstrated by our previous work. Nicotine (2.0 mg/kg.d) or saline was injected subcutaneously into pregnant rats every morning from gestational day (GD) 9 to 20. Fetal femurs or tibias were harvested for analysis on GD 20. We found massive accumulation of hypertrophic chondrocytes and a delayed formation of primary ossification center (POC) in the fetal femur or tibia of rat fetus after PNE, which was accompanied by a decreased amount of osteoclasts in the POC and up-regulated expression of osteoprotegerin (OPG) but by no obvious change in the expression of receptor activator of NF-κB ligand (RANKL). In primary osteoblastic cells, both nicotine (0, 162, 1620, 16,200 ng/ml) and corticosterone (0, 50, 250, 1250 nM) promoted the mRNA expression of OPG but concentration-dependently suppressed that of RANKL. Furthermore, blocking α4β2-nicotinic acetylcholine receptor (α4β2-nAChR) or glucocorticoid receptor rescued the above effects of nicotine and corticosterone, respectively. In conclusion, retarded osteoclastogenesis may contribute to delayed endochondral ossification in long bone in fetal rats with PNE. The adverse effects of PNE may be mediated via the direct effect of nicotine and indirect effect of maternal corticosterone on osteoblastic cells.” (Hu et al. 2018).

“Perinatal nicotine exposure can not only lead to lung dysplasia in offspring, but also cause epigenetic changes and induce transgenerational asthma. Previous studies have shown that electro-acupuncture (EA) applied to "Zusanli" (ST 36) can improve the lung morphology and correct abnormal expression of lung development-related protein in perinatal nicotine exposure offspring. However, it is still unclear whether ST 36 has a specific therapeutic effect and how maternal acupuncture can protect the offspring from pulmonary dysplasia. In this study, we compared the different effect of ST 36 and "Fenglong" (ST 40), which belong to the same meridian, in terms of lung pulmonary function and morphology, PPARγ, β-catenin, GR levels in the lung tissues and CORT in the serum of perinatal nicotine exposure offspring, and explored the mechanism of acupuncture based on the maternal hypothalamus-pituitary-adrenal (HPA) axis. It is shown that EA applied to ST 36 could restore the normal function of maternal HPA axis and alleviate maternal glucocorticoid overexposure in offspring, thereby it can up-regulate the PTHrP/PPARγ and down-regulate the Wnt/β-catenin signaling pathways, and protects perinatal nicotine exposure-induced pulmonary dysplasia in offspring. Its effect is better than that of ST 40. These results are of great significance in preventing perinatal nicotine exposure-induced pulmonary dysplasia in offspring.” (Liu Y et al. 2018).

“The objective of the current study is to test the hypothesis that the deletion of alpha(α)2\* nicotinic acetylcholine receptors (nAChRs) (encoded by the Chrna2 gene) ablate maternal nicotine-induced learning and memory deficits in adolescent mice. We use a pre-exposure-dependent contextual fear conditioning behavioral paradigm that is highly hippocampus-dependent. Adolescent wild type and α2-null mutant offspring are exposed to vehicle or maternal nicotine exposure (200 μg/ml, expressed as base) in the drinking water throughout pregnancy until weaning. Adolescent male offspring mice are tested for alterations in growth and development characteristics as well as modifications in locomotion, anxiety, shock-reactivity and learning and memory. As expected, maternal nicotine exposure has no effects on pup number, weight gain and only modestly reduces fluid intake by 19%. Behaviorally, maternal nicotine exposure impedes extinction learning in adolescent wild type mice, a consequence that is abolished in α2-null mutant mice. The effects on learning and memory are not confounded by alternations in stereotypy, locomotion, anxiety or sensory shock reactivity. Overall, the findings highlight that the deletion of α2\* nAChRs eliminate the effects of maternal nicotine exposure on learning and memory in adolescent mice.” (Mojica et al. 2018).

“Use of tobacco products is injurious to health in men and women. However, tobacco use by pregnant women receives greater scrutiny because it can also compromise the health of future generations. More men smoke cigarettes than women. Yet the impact of nicotine use by men upon their descendants has not been as widely scrutinized. We exposed male C57BL/6 mice to nicotine (200 μg/mL in drinking water) for 12 wk and bred the mice with drug-naïve females to produce the F1 generation. Male and female F1 mice were bred with drug-naïve partners to produce the F2 generation. We analyzed spontaneous locomotor activity, working memory, attention, and reversal learning in male and female F1 and F2 mice. Both male and female F1 mice derived from the nicotine-exposed males showed significant increases in spontaneous locomotor activity and significant deficits in reversal learning. The male F1 mice also showed significant deficits in attention, brain monoamine content, and dopamine receptor mRNA expression. Examination of the F2 generation showed that male F2 mice derived from paternally nicotine-exposed female F1 mice had significant deficits in reversal learning. Analysis of epigenetic changes in the spermatozoa of the nicotine-exposed male founders (F0) showed significant changes in global DNA methylation and DNA methylation at promoter regions of the dopamine D2 receptor gene. Our findings show that nicotine exposure of male mice produces behavioral changes in multiple generations of descendants. Nicotine-induced changes in spermatozoal DNA methylation are a plausible mechanism for the transgenerational transmission of the phenotypes. These findings underscore the need to enlarge the current focus of research and public policy targeting nicotine exposure of pregnant mothers by a more equitable focus on nicotine exposure of the mother and the father.” (McCarthy et al. 2018).

“Prenatal nicotine exposure (PNE) can cause hypersensitivity of hypothalamic-pituitary-adrenal (HPA) axis in offspring with intrauterine growth retardation. The purpose of this study was to explore the original mechanism of intrauterine development that mediates hypersensitivity of the HPA axis in offspring due to PNE. Pregnant Wistar rats were injected subcutaneously with 2 mg/kg·d of nicotine on the 9th to the 20th gestational day (GD9-GD20) and the fetuses were extracted at GD20. Compared with the control group, fetal rats by PNE showed increased hippocampal apoptosis, reduced synaptic plasticity and downregulation of the brain-derived neurotrophic factor (BDNF) pathway, whereas glutamic acid decarboxylase 67 (GAD67) expression was upregulated. Rat fetal hippocampal H19-7/IGF1R cell lines were treated with different concentrations of nicotine (1, 10 and 100 μM) for 3 days, the extracellular fluid glutamate (Glu) level increased and similar effects were observed as in vivo. Intervention treatments caused the opposite results. These results indicated that PNE downregulates the BDNF pathway and mediates the hippocampal excitotoxicity; then, the compensatory upregulation of GAD67 causes the imbalance of signal output in the fetal hippocampus. The negative feedback regulation of the paraventricular hypothalamic nucleus by the hippocampus is unbalanced, eventually causing hypersensitivity of the HPA axis of the offspring.” (Pei et al. 2019).

“Clinical study showed that smoking during pregnancy deceased the thymus size in newborns. However, the long-term effect remains unclear. This study was aimed to observe the effects of prenatal nicotine exposure (PNE) on the development of thymus and the T-lymphocyte subpopulation in mice offspring from the neonatal to adulthood. Both the thymus weight and cytometry data indicated that PNE caused persistent thymic hypoplasia in male offspring from neonatal to adult period and transient changes in female offspring from neonatal to prepuberal period. Flow cytometry analysis disclosed a permanent decreased proportion and number of mature CD4 single-positive (SP) T cells in thymus of both sex. In addition, the PNE male offspring showed a more serious thymus atrophy in the ovalbumin (OVA)-sensitized model. Moreover, increased autophagic vacuole and elevated mRNA expression of Beclin 1 were noted in PNE fetal thymus. In conclusion, PNE offspring showed thymus atrophy and CD 4 SP T cell reduction at different life stages. Mechanically, PNE induced excessive autophagy in fetal thymocytes might be involved in these changes. All the results provided evidence for elucidating the PNE-induced programmed immune diseases.” (Qu et al. 2019).

“Our previous studies showed that paternal nicotine exposure can lead to hyperactivity in the offspring. Nevertheless, the cross-generational effects of maternal and biparental nicotine exposure remain unclear. In this study, female and male mice were exposed respectively by nicotine before pregnancy. The maternal pre-pregnancy nicotine exposure led to depression-like behaviors in the F1 offspring. However, after biparental pre-pregnancy nicotine exposure, seventy percentage of the offspring exhibited a depressive phenotype while 20% were hyperactive, and the remaining exhibited no obvious abnormal behavior. The cross-generational effects appeared to be mediated via disruption of the balance between GSK3 and p-GKS3 by nicotine. These results suggested that pre-pregnancy nicotine exposure can induce alterations in the behavior of the offspring, and the cross-generational effects of maternal nicotine exposure were particularly serious.” (Zhang M et al. 2018).

“In a prenatal study, rat pups exposed to either nicotine (6 mg/kg/day) or saline in utero (osmotic mini pump) and then were chronically instrumented with ECG electrodes for measurements of heart rate (HR) and respiratory frequency (RF) on postnatal days (P) 13, 16 and 26. No significant effect of prenatal nicotine exposure (PNE) on pups’ weight was identified. In males there was no significant effect of age or treatment on resting RF, but there was a strong trend for resting HR to be elevated at rest in PNE versus control pups. Alternatively, in females, independent of treatment, RF was significantly lower at P26 compared to both P13 and P16. No parallel effect of age or PNE on HR was identified in the females. Additionally, alternations in cardiorespiratory integration appeared to persist into later development in males only, potentially increasing the risk for cardiovascular diseases such as hypertension later in life.” (Boychuk and Hayward 2011).

“Female Lister hooded rats were exposed to nicotine solutions both before and during pregnancy. These females were divided into groups consuming solutions of different concentrations 0.03-0.12 mg/ml (2.7-7 mg/kg). Animals were divided into groups consuming solutions of different concentrations such that animals that initially consumed the solutions most readily were exposed to progressively higher concentrations. Offspring were evaluated in a test battery measuring maturational and developmental milestones. Solutions with concentrations below 0.06 mg/ml were well tolerated with some moderate adverse effects at the highest dose. Concentrations above 0.08 mg/ml led to a large drop in fluid consumption and body weight. Strong teratogenic effects of prenatal nicotine exposure were observed at concentrations above 0.06 mg/ml, including developmental and maturational delays shown by measures of pinnae detachment, fur appearance, incisor eruption, eye opening and righting reflex. Negative geotaxis, grip strength and weight gain were impaired and postnatal mortality was increased. The present study clearly shows teratogenic effects, however, it suffers from a number of limitations, including drop in water consumption associated with vasoconstriction and hypoxia leading possibly to teratogenic effects. Secondly, the method for assigning animals to particular nicotine solutions, based on their actual consumption of and tolerance to nicotine’s effects, might influence and to some extent confound the dose-related nature of the teratogenic effects.” (Schneider et al. 2010).

“Pregnant Sprague Dawley rats (n=56) were anesthetized on gestation day 2 and osmotic mini pumps were placed in the interscapular region diffusing either saline or nicotine bitartrate (2 or 6 mg/kg/day). Dams were sacrificed at term (21 days) in all nicotine and saline groups. Blood analysis including plasma nicotine, serum corticosterone and hematocrit count were performed on several gestational days (GD 4, 16 and 21. Maternal plasma nicotine in 6 mg/kg/day group did not show any differences between GD 4 and 16 and showed a marginal decrease on GD 21. No difference in plasma nicotine was observed between GD 4 and GD 16 or GD 16 and GD 21 in a 2 mg/kg/day group. The maternal weight in all groups infused with nicotine and saline increased with advancing pregnancy. No significant differences were observed in daily weight gain as well in final maternal weight at term gestation among all the groups. Maternal hematocrit was not affected by nicotine administration. The number of pups per litter, pup weight, placental weight and weight of various pups’ organs was not significantly affected by any dose of nicotine as compared with the saline infused animals. The plasma corticosterone concentrations were higher on GD 4 as compared with GD 21 in both nicotine (6 mg/kg/day) and saline group. However, no difference was observed in plasma corticosterone between these two groups. Overall, the nicotine treatment did not result in relevant adverse effect in the offspring.” (Hussein et al. 2007).

“In another study, pregnant Sprague-Dawley rats (n=5 per group) were given 0, 15 or 25 mg nicotine pellets (21-day time release) throughout pregnancy. Some offspring continued to receive 1 or 2 mg/kg bw /day during postnatal period. The remaining offspring received no further treatment after birth. Nicotine treatment did not reveal a significant effect on maternal body weight gain or on the number of pups and their gender compared to the control group. The body weight of all offspring was monitored until adulthood. Regardless of the timing of nicotine exposure, the nicotine treatment significantly increased the body weight in female offspring starting on postnatal day (PD) 35 and such increase persisted into adulthood (PD 91). However, nicotine exposure paradigm led to a transient increase in male offspring body weight on PD 35. Additionally, the neonatal thyroid status from all treatment groups was assessed from the serum of 10-day-old pups, showing no difference in total T4 level, T3 uptake and the calculated Free T4 index." (Chen and Kelly 2005).

“Nicotine-delivering transdermal patches were applied to the back of pregnant Sprague-Dawley rats (n=2-13/group) either during entire pregnancy (2-19 days) or during the first trimester (2-7 days) of the gestation period. Control animals received either no treatment or placebo patch. Pregnancy failure was 100% in animals exposed to 3.5 mg of nicotine per day and ca. 50% in animals exposed to 1.75 mg per day during the entire pregnancy. Exposure to nicotine only during the first trimester of pregnancy had a less dramatic effect and pregnancy failure was statistically significant only in animals exposed to 3.5 mg nicotine per day. At this dose, the effect was seen even if the patch was applied through days 3 to 5, suggesting that nicotine has a pre-implantation effect. Analysis of litter size did not show any significant differences between different groups, including the sham-handled controls.” (Witschi et al. 1994).

“In an older study, female Swiss-Webster mice were given nicotine in drinking water for at least 2 weeks before breeding and throughout pregnancy. At first, animals in the nicotine-treated groups were given nicotine at a concentration of 20 μg/ml. After 7 days the concentration was increased to 60 μg/ml for two of the groups, and after an additional 14 days increased to 100 pg/ml for one group. The approximate daily dose of nicotine in the mice receiving 20, 60, and 100 μg/ml solutions were 5 .7, 17.2, and 28 .6 mg/kg, respectively. Control mice received water without nicotine. No signs of maternal toxicity were reported. The fetuses and placentas of all animals were examined on the 17th day of gestation. There was no reduction in the litter size in the treated animals, however, fetuses of animals treated with 60, and 100 μg/ml weighted up to 12% less than control animals. Additionally, the mean placental weight and the level of the amino acid α-aminoisobutyric acid in the placentas were decreased in high-dose animals compared with controls. Nicotine treatment had no effect on the placental concentrations of acetylcholine or the activities of acetylcholinesterase or acetyltransferase. The NOAEL for embryotoxic effects was 5.7 mg/kg bw/day.” (Rowell and Clark, 1982).

Effects on fertility

“The activation of nicotinic cholinergic receptors (nAChR) inhibits the reproductive axis; however, it is not clear whether nicotine may directly modulate the release of hypothalamic gonadotropin-releasing hormone (GnRH). Experiments carried out in GT1-1 immortalized GnRH neurons reveal the presence of a single class of high affinity α4β2 and α7 nAchR subtypes. The exposure of GT1-1 cells to nicotine does not modify the basal accumulation of GnRH. However, nicotine was found to modify GnRH pulsatility in perifusion experiments and inhibits, the release of GnRH induced by prostaglandin E1 or by K+-induced cell depolarization; these effects were reversed by D-tubocurarine and α-bungarotoxin. In conclusion, the results reported here indicate that: functional nAChRs are present on GT1-1 cells, the activation of the α-bungarotoxin-sensitive subclass (α7) produces an inhibitory effect on the release of GnRH and that the direct action of nicotine on GnRH neurons may be involved in reducing fertility of smokers.” (Messi et al. 2018).

“BACKGROUND: Several studies demonstrate that cigarette smoking has a negative effect on the reproductive health of both genders. The mechanisms by which it alters male gonadic function are not entirely clear. The combustion of cigarette produces a lot of chemical compounds that may be responsible for the negative impact of cigarette smoke on sperm parameters. In particular, the effects on semen of nicotine, a substance present in the tobacco plant and the main constituent of cigarette smoke, have been studied, showing that this alkaloid alters sperm parameters. Recently we investigated the mechanism by which nicotine damages sperm through the evaluation of the expression of nicotinic receptors subunits in human spermatozoa. CONCLUSION: 8 nAChR subunits found to date in mammals are expressed in human spermatozoa but, in non-smokers subjects, only α7 subunit is translated. Cigarette smoking may stimulate the expression of some subunits, not translated in non-smokers. Therefore, the presence in sperm of other nAChR subunits than α7 could represent a marker for smoking-related sperm damage.” (Condorelli et al. 2018).

“A new report has shown that nicotine exposure can decrease serum testosterone by apoptosis in Leydig cells; however, in our previous studies, we have almost never observed apoptosis there. The purpose of this study is to ensure whether apoptosis or autophagy in Leydig cells occurred. Our results confirmed again that the concentration of testosterone in the sera of nicotine-treated mice statistically decreased (P < 0.05). Furthermore, the data of single cell transcriptome indicated that the expression of autophagy-related genes was increased after nicotine exposure. Likewise, chemical and immune-histological staining demonstrated that autophagy of the Leydig cells increased after nicotine treatment rather than apoptosis. Apoptosis mainly exists in spermatids. Further, the expression of autophagy-related genes, such as Beclin1 and LC3, were up-regulated after nicotine exposure (P < 0.05). Additionally, the data of transmission electron microscopy showed more autophagosomes in the Leydig cells of the nicotine-exposed groups than the cells of the control groups. Moreover, immunofluorescent staining of LC3 in the TM3 Leydig cell line indicated that rapamycin and nicotine exposure up-regulates the autophagy phenotype/process and down-regulates their testosterone synthesis. In addition, the methylation level of the promoter region of TCL1 is increased in the nicotine-treated group compared to the control group, consequently decreasing the expression of TCL1. In conclusion, the autophagy in Leydig cells induced by nicotine, which is set by the hyper-methylation of the TCL1 promoter region via the TCL1-mTOR-autophagy signaling pathway.” (Zhao et al. 2018).

“Exposure to nicotine in smoking contributes to most unexplained male infertility but the mechanisms remain to be fully elucidated. Zinc (Zn) is an essential trace element in normal growth, development and reproduction. Zinc oxide nanoparticles (ZnONPs) are well-known antioxidants. Therefore, this work was designed to investigate the potential ability of ZnONPs to protect testis and epididymis in nicotine-treated rats. Forty adult male Wistar albino rats were divided into control group and two experimental groups (treated and supplemented rats). In the treated group, rats received nicotine at a dose of 1 mg/kg/day orally for30 days. Rats in the supplemented group received ZnONPs (10 mg/kg/day) with nicotine (1 mg/kg/day), orally for the same period. Testicular and epididymal sections were stained with H&E to assess the histological changes. Negrosin-eosinstaining of epididymal sperms was performed to assess their viability and morphological changes. Serum testosterone, FSH and LH levels were assessed. Also, oxidative stress parameters and semiquantitative real-time PCR for steroidogenic enzymes were measured. Morphometric studies of both organs were statistically analyzed. Mild to severe testicular and epididymal structural changes together with sperm morphological abnormalities were detected in nicotine-treated rats. Biochemical results also showed a decrease in all measured parameters except for an increased malondialdehyde (MDA) level that meant deterioration of their reproductive function. On the other hand, ZnONP supplementation in the last group showed an obvious improvement in all investigated parameters.” (Mohamed and Abdelrahman 2019).

“Male albino mice were intraperitoneally administered 0, 2, 4 and 6 mg/kg of nicotine for 15 days. The treatment has caused a significant decrease in the weight of testis, reduced number of spermatocytes and spermatids, but increased the number of spermatogonia, which may be due to reduced conversion to subsequent stages. These changes were explained by the non-availability of pituitary gonadotropins essential for initiation and completion of spermatogenesis and steroidogenesis in the testis due to the administration of nicotine being CNS depressor and inhibiting neural stimulus essential for release of pituitary gonadotropins.” (Reddy 1998).

“Groups of male Swiss albino mice (n=12/group) received nicotine in drinking water at doses equivalent to 0 or 2.7 mg/kg bw/day for 7 weeks or 0 or 2.3 mg/kg bw/day for 20 weeks. When females were mated with males at 1 to 4 weeks after the end of the 7-week nicotine exposure, the numbers of litters and offspring were similar compared with the control group. Additionally, no congenital abnormalities were detected. However, offspring of females mated with males at 1 to 6 weeks after the end of 20-week exposure, showed a statistically significant increase in the incidence of abnormalities of the limbs in the first and second week after nicotine treatment of male mice.” (Hemsworth 1981).

“There has been much concern about the use of EC by pregnant women and the role that nicotine may play in harming foetal development. Animal research has suggested foetal exposure to very high doses of nicotine has adverse consequences which are maintained through to adolescence, but the relevance for humans is unclear (101, 102).

In humans, it has been difficult to separate the impact of nicotine from smoking in pregnancy, given the low use of cleaner nicotine products among pregnant women. Thus assumptions about harms from nicotine in human pregnancies, have until recently, emerged either as a result of studies of tobacco use in pregnancy or are extrapolated from animal research. More recently, however, it has been reported that infants born to pregnant smokers, who used NRT for smoking cessation during pregnancy, were less likely to have developmental impairments compared with those who used placebo two years after birth (103). The reason for this requires more research but the authors argued it could be due to reduced smoking early in pregnancy as a consequence of NRT use. The licence for prescribing NRT was extended in the UK in 2005 to include use in pregnancy and NRT is currently widely prescribed in the UK to pregnant women who smoke (104).

Limited research has been conducted with pregnant smokers or ex-smokers who use EC (105). Further research is needed and a large trial of EC for smoking cessation in pregnant women is now underway in the UK (<https://www.journalslibrary.nihr.ac.uk/programmes/hta/155785/#/)>.” (Public Health England, 2018)

“Impaired placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) activity which inactivates maternal glucocorticoids is associated with poor fetal growth and a higher risk of chronic diseases in adulthood. This study aimed to elucidate the epigenetically regulatory mechanism of nicotine on placental 11β-HSD2 expression. Pregnant Wistar rats were administered 1.0 mg/kg nicotine subcutaneously twice a day from gestational day 9 to 20. The results showed that prenatal nicotine exposure increased corticosterone levels in the placenta and fetal serum, disrupted placental morphology and endocrine function, and reduced fetal bodyweight. Meanwhile, histone modification abnormalities (decreased acetylation and increased di-methylation of histone 3 Lysine 9) on the HSD11B2 promoter and lower-expression of 11β-HSD2 were observed. Furthermore, the expression of nicotinic acetylcholine receptor (nAChR) α4/β2, the phosphorylation of extracellular regulated kinase 1/2 (ERK1/2) and Ets-like protein-1 (Elk-1), and the expression of early growth response-1 (Egr-1) were increased in the nicotine groups. In human BeWo cells, nicotine decreased 11β-HSD2 expression, increased nAChRα9 expression, and activated ERK1/2/Elk-1/Egr-1 signaling in the concentration (0.1-10 μM)-dependent manner. Antagonism of nAChRs, inhibition of ERK1/2 and Egr-1 knockdown by siRNA were able to block/abrogate the effects of nicotine on histone modification and expression of 11β-HSD2. Taken together, nicotine can impair placental structure and function, and induce fetal developmental toxicity. The underlying mechanism involves histone modifications and down-regulation of 11β-HSD2 through nAChRs/ERK1/2/Elk-1/Egr-1 signaling, which increases active glucocorticoids levels in the placenta and fetus, and eventually inhibits the fetal development.” (Zhou et al. 2018).

“OBJECTIVE: This study aims at evaluating the endometrial receptivity in uterus of pregnant rats exposed to nicotine via examination of integrin expression by immunohistochemical effect. METHODS: In this study, 16 healthy pregnant rats were divided into two groups of control and study groups each comprising eight rats. The rats randomised to study group were given a certain amount of nicotine before and during the pregnancy. Integrin expression was detected in uterus of all rats by immunohistochemical staining. The effect of nicotine exposure on embryo implantation and the endometrial receptivity were immunohistochemically and pathologically evaluated. RESULTS: Comparison of both groups revealed no difference in living, viable foetuses. Intensity and universality of immunohistochemical staining of Integrin β3 for endometrial epithelium and endometrial stroma were detected to be identical between the groups. CONCLUSION: No immunochemical effect was observed on integrin expression, which is a very important part of receptivity in an animal model created with pregnant rats that were transdermally exposed to nicotine. Our study demonstrated that the harmful effect of nicotine use before and pregnancy on implantation is limited at the level of integrin expression, in a dose-dependent manner and also by considering the method of administration.” (Akpak et al. 2017).

“Background: Nicotine is a major toxic and hazardous component of cigarette smoke, and it has been widely used in nicotine replacement therapy (NRT). This study was aimed to investigate the effects of chronic low-dose nicotine on sperm characteristics and reproductive organ integrity in adolescent male Sprague-Dawley rats. Methods: Twelve rats were equally divided into two groups. Group I received normal saline, and group II received 0.6 mg/kg body weight nicotine intraperitoneally for 28 consecutive days. At the end of the experimental period, sperm was collected for sperm characteristic evaluation, and the testes and prostate were isolated for biochemical and morphological analysis. The effects of nicotine on the body and reproductive organ weights of the animals were evaluated. Results: Chronic nicotine treatment significantly (P < 0.05) altered the sperm count, motility, viability, and morphology, and remarkably increased the malondialdehyde (P < 0.001) and advanced oxidation protein product (P < 0.05) levels in the testes and prostate of nicotine-treated group compared to control group. Moreover, nicotine caused a significant decrease (P < 0.05) in the superoxide dismutase activity of the testes. No significant differences were observed in the reduced glutathione level in both of the testes and prostate of nicotine group compared with control group. Nicotine also induced histopathological alteration in the testes. Conclusion: A low-dose nicotine exposure at 0.6 mg/kg caused detrimental effects on sperm characteristics and induced oxidative stress in the testes and prostate.” (Budin et al. 2017).

“BACKGROUND: Osteoarthritis (OA) is a chronic joint disease characterized by a progressive and irreversible degeneration of articular cartilage. Among the environmental risk factors of OA, tobacco consumption features prominently, although, there is a great controversy regarding the role of tobacco smoking in OA development. Among the numerous chemicals present in cigarette smoke, nicotine is one of the most physiologically active molecules. OBJECTIVE: The aim of the study was (i) to measure the impact of nicotine on the proliferation and chondrogenic differentiation of mesenchymal stem cells from the human Wharton's jelly (hWJ-MSCs) into chondrocytes, (ii) to investigate whether the α7 nicotinic acetylcholine receptors (nAChRs) was expressed in hWJ-MSCs and could play a role in the process. The project benefits from the availability of an umbilical cord bank from which hWJ-MSCs were originated. METHODS: The hWJ-MSCs were cultured and used up to passage 5. The proliferation of hWJ-MSCs with 5 μM nicotine was measured by the MTT assay on the 1st, 2nd, 3rd, and 6th day. Flow cytometry analysis was used to detect cell apoptosis/necrosis by Annexin V/PI double-staining. The chondrogenic differentiation grade of hWJ-MSCs induced by TGFβ3 was assessed by the Sirius red and Alcian blue staining. The expression of markers genes was followed by quantitative real-time PCR. The expression of nAChRs was followed by RT-PCR. The functional activity of α7 nAChR was evaluated by calcium (Ca2+) influx mediated by nicotine using the Fluo-4 NW Calcium assay. RESULTS: The proliferation of hWJ-MSCs was significantly impaired by nicotine (5 μM) from the 3rd day of treatment, but nicotine did not significantly induce modifications on the viability of hWJ-MSCs. Alcian blue staining indicated that the amount of proteoglycan was more abundant in control group than in the nicotine group, but no difference was observed on the total collagen amount using Sirius red staining. The mRNA expression of Sox9, type II collagen (Col2a1), aggrecan in control group was higher than in the nicotine group. We found that hWJ-MSCs expressed α7 nAChR. The receptor agonist nicotine caused calcium (Ca2+) influx into hWJ-MSCs suggesting that the calcium ion channel α7 homopolymer could mediate this response. CONCLUSIONS: At the concentration used, nicotine had an adverse effect on the proliferation and chondrogenic differentiation of hWJ-MSCs which was probably impaired through a α7 nAChR mediation.” (Yang et al. 2017).

“Maternal cigarette smoke, including prenatal nicotinic exposure (PNE), is responsible for sudden infant death syndrome (SIDS). The fatal events of SIDS are characterized by severe bradycardia and life-threatening apneas. Although activation of transient receptor potential vanilloid 1 (TRPV1) of superior laryngeal C fibers (SLCFs) could induce bradycardia and apnea and has been implicated in SIDS pathogenesis, how PNE affects the SLCF-mediated cardiorespiratory responses remains unexplored. Here, we tested the hypothesis that PNE would aggravate the SLCF-mediated apnea and bradycardia *via* up-regulating TRPV1 expression and excitation of laryngeal C neurons in the nodose/jugular (N/J) ganglia. To this end, we compared the following outcomes between control and PNE rat pups at postnatal days 11-14: *1*) the cardiorespiratory responses to intralaryngeal application of capsaicin (10 µg/ml, 50 µl), a selective stimulant for TRPV1 receptors, in anesthetized preparation; *2*) immunoreactivity and mRNA of TRPV1 receptors of laryngeal sensory C neurons in the N/J ganglia retrogradely traced by 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate; and *3*) TRPV1 currents and electrophysiological characteristics of these neurons by using whole-cell patch-clamp technique *in vitro* Our results showed that PNE markedly prolonged the apneic response and exacerbated the bradycardic response to intralaryngeal perfusion of capsaicin, which was associated with up-regulation of TRPV1 expression in laryngeal C neurons. In addition, PNE increased the TRPV1 currents, depressed the slow delayed rectifier potassium currents, and increased the resting membrane potential of these neurons. Our results suggest that PNE is capable of aggravating the SLCF-mediated apnea and bradycardia through TRPV1 sensitization and neuronal excitation, which may contribute to the pathogenesis of SIDS.” (Gao et al. 2017).

“Epidemiological studies show that maternal cigarette smoking is associated with an increased risk of cardiovascular diseases in postnatal life. Baroreflex sensitivity (BRS) is an important index for evaluating the homeostasis of the cardiovascular system. This experiment was designed to investigate the possible mechanism of prenatal nicotine on the adult male offspring's heart rate (HR) increase due to BRS. Pregnant rats received the 0.3 ml of saline or nicotine (1.5 mg kg-1) by subcutaneous injection from gestational days 3 to 21. The male offsprings of saline injected dams were the control group, and the male offsprings of the nicotine injected dams were the nicotine group. The 90-day-old male offsprings' funny current (I f) of their sinoatrial node (SAN) cells, BRS induced by intravenous injection of angiotensin (Ang) II in the presence or absence of the L-nitro-arginine methylester (L-NAME), cervical vagal activity, c-fos protein levels of the cervical spinal cord-8 to the thoracic spinal cord-5 (C8-T5) lateral horn neuron, and blood hormones were tested. The results showed that prenatal nicotine exposure had no effect on the offsprings' I f of their SAN cells, but it significantly decreased the offsprings' BRS. The c-fos protein levels of the C8-T5 lateral horn neurons and the blood catecholamine levels were increased in the nicotine group, but the cervical vagal activity was not changed. After intraventricular injection of L-NAME, the nicotine exposed offsprings' BRS was partly recovered. These data suggest that prenatal nicotine exposure results in hyper reactivity of the spinal sympathetic nerve center and a higher peripheral catecholamine hormone state of 90-day-old male offsprings, and these may be the reason for the BRS inhibition and HR increase. Nitric oxide (NO) may participate in the process acting as an important neurotransmitter.” (Yu et al. 2017).

## *5.4.* *Mutagenicity*

**In vitro**

Nicotine has been shown to be give negative response in S. typhimurium (TA97, TA98, TA100, TA1535 and TA 1537) assay up to 5000 µg/plate both with and without metabolic activation (S9) (Riebe et al. 1982; de Flora et al. 1984; Brams et al. 1987).

Additionally, nicotine and its four major metabolites including cotinine, nicotine-N’-oxide, cotinine-N-oxide and trans-3’hydroxycotinine were evaluated for mutagenic and genotoxic potential in S. typhimurium (TA98, TA100, TA1535 and TA 1537) assay at concentrations ranging from 0-1000 µg/plate and in the CHO sister-chromatid exchange (SCE) assay up to 1000 µg/ml. All the assays were conducted with and without S9 metabolic activation. None of the five compounds increased the frequency of mutations and SCEs indicating that neither of the studied substances was mutagenic nor genotoxic (Doolittle et al. 1995).

Furthermore, nicotine and its metabolites (cotinine, trans-3’hydroxycotinine and nicotine-N’-oxide, cotinine-N-oxide) were also investigated in S. typhimurium (TA100, TA7004, TA7005 and TA7006) at the concentrations up to 1000 µg/plate, with and without the metabolic activation (S9). None of the tested substances showed the missense back mutation in two of the tested strains of S. typhimurium (TA100 and TA7004) (Yim and Que Hee, 1995).

Trivedi et al., (1990) reported that nicotine at concentrations ranging from 150-625 µg/ml, increased the the frequency of chromosomal aberrations in CHO cells. However, the reported increase was arrived at by incorporating chromosome and chromatid gaps into the primary statistical analysis, even though the OECD international guidelines (1983) recommended that gaps or other achromatic lesions not to be included when calculating chromosome aberration frequency, since they may result from staining artifacts. As Trivedi et al., indicated, if gaps were excluded, nicotine would not have increased the frequency of chromosomal aberrations following either 2 or 4-hour treatment, and only slightly increased the frequency of chromosome aberrations following 24-hour continuous treatment.

In a DNA repair assay with E.coli pol A+/polA- nicotine at 10 µg induced reparable DNA damage while its metabolites nicotine 1’-N-oxide (400 µg) and cotinine (800 µg) gave negative results (Riebe M et al., 1982). However, de Flora et al., (1984) found nicotine negative in DNA repair assay with E.coli pol A+/polA.

In a bacterial assay with Vibrion fischeri strain NRRL-B-11177, cotinine gave positive response in the absence of S9 at 1.25 mg/ml (9-15 h incubation) and at 2.50 mg/ml (18-30 incubation hours) signifying potential mutagenicity and teratogenicity. In the presence of S9, cotinine was positive at 1.25 mg/ml after 9 incubation hours. In contrast, nicotine was negative at any concentration or incubation time. Nicotine/cotinine mixtures were still positive at physiological concentrations, with potentiation relative to cotinine alone with and without S9 (Yim and Que Hee, 1995).

**In vivo**

In a conventional mouse bone marrow assay, single doses of nicotine (1 or 2 mg/kg bw) were administered by oral intubations and bone marrow was sampled at 24 hours (1 mg/kg bw) or at 6, 12 and 18 hours after treatment (2 mg/kg bw). Nicotine treatment did not increase the micronucleus frequency in polychromatic erythrocytes while the positive control (mitomycin C) yielded the expected result (Adler and Attia 2003).

This data contradicts another in vivo study by Sen (1991), where 0.77 and 1.1 mg/kg by nicotine gavage administration induced the chromosomal aberrations in Swiss albino mouse bone marrow cells at all sampling intervals (6, 12, 18 and 24 hours after the treatment) compared to the control animals receiving isotonic saline.

In another study by Attia (2007), Swiss albino female and male mice received 4, 8 or 16 mg/kg bw of nicotine and were sacrificed at 18, 24, 30, 36 and 48 hours after dosing. Despite the evident signs of acute toxicity shown by the animals, mainly at the two highest doses, nicotine administration did not induce micronucleated polychromatic erythrocytes (MCPCE) in bone marrow at either of the sampling times, 18 and 24 hours. At the 30 and 36 h sampling times, nicotine (8 and 16 mg/kg) significantly increased MNPCE frequencies in both genders. This was accompanied by a significantly greater bone-marrow toxicity in both male and female mice. By the 48 hours, the recovery was observed even though the cytotoxicity was high. These findings indicate that nicotine at high doses and after prolonged time intervals is genotoxic and cytotoxic to mouse bone marrow.

Nicotine (compounds related to) (CAS RN 54-11-5) was not mutagenic in a bacterial mutagenicity test in Salmonella typhimurium strains TA97, TA98, TA100 and TA1535, when aqueous solutions were tested at up to 10 mg/plate, with and without S9 (the highest dose was toxic in some strains). (NTP, undated).

“E-cigarette smoke delivers stimulant nicotine as aerosol without tobacco or the burning process. It contains neither carcinogenic incomplete combustion byproducts nor tobacco nitrosamines, the nicotine nitrosation products. E-cigarettes are promoted as safe and have gained significant popularity. In this study, instead of detecting nitrosamines, we directly measured DNA damage induced by nitrosamines in different organs of E-cigarette smoke-exposed mice. We found mutagenic O6-methyldeoxyguanosines and γ-hydroxy-1*,N2* -propano-deoxyguanosines in the lung, bladder, and heart. DNA-repair activity and repair proteins XPC and OGG1/2 are significantly reduced in the lung. We found that nicotine and its metabolite, nicotine-derived nitrosamine ketone, can induce the same effects and enhance mutational susceptibility and tumorigenic transformation of cultured human bronchial epithelial and urothelial cells. These results indicate that nicotine nitrosation occurs in vivo in mice and that E-cigarette smoke is carcinogenic to the murine lung and bladder and harmful to the murine heart. It is therefore possible that E-cigarette smoke may contribute to lung and bladder cancer, as well as heart disease, in humans.” (Lee et al. 2018).

## *5.5.* *Cytotoxicity*

“It is well-documented that nicotine, the main active ingredient in cigarettes, results in endothelial cell injury in numerous diseases. However, whether nicotine plays a crucial role in endothelial cell injury in diabetes and the exact molecular mechanism that mediates this process have not been fully elucidated. The current study aimed to investigate the effects of nicotine on endothelial cell injury in diabetes and the specific molecular mechanism by which it plays a role. Human umbilical vein endothelial cells (HUVECs) were incubated in HG/HF media and treated with nicotine, PYR-41 (a selective ubiquitin E1 inhibitor), Akt-overexpressing adenovirus, or TTC3 and MUL1 shRNA adenovirus. Cell viability was subsequently detected by the CCK8 assay, and apoptosis was examined by caspase-3 cleavage and activity analysis. Compared to the HG/HF incubated group, nicotine incubation significantly decreased cell survival and increased apoptosis. Moreover, nicotine induced Akt degradation via UPS, and Akt overexpression blocked nicotine-induced apoptosis in HUVECs cultured in HG/HF media. Furthermore, the TTC3 and MUL1 shRNA adenovirus dramatically decreased the Akt ubiquitination and apoptosis induced by nicotine. These results indicate that nicotine-induced Akt ubiquitination and degradation occurs through TTC3 and MUL1 and results in a dramatic increase in apoptosis in HUVECs cultured in HG/HF media.” (Cao et al. 2018).

“Tobacco heating products (THPs) represent a subset of the next-generation nicotine and tobacco product category, in which tobacco is typically heated at temperatures of 250-350 °C, thereby avoiding many of the harmful combustion-related toxicant emissions of conventional cigarettes. In this study, we have assessed aerosol generation and cytotoxicity from two commercially available THPs, THP1.0 and THS, relative to tobacco smoke from 3R4F reference cigarettes, using an adapted Borgwaldt RM20S Smoking Machine. Quantification of nicotine in the exposed cell-culture media showed greater delivery of nicotine from both THPs than from the cigarette. Using Neutral Red Uptake assay, THPs demonstrated reduced in vitro cytotoxicity in H292 human bronchial epithelial cells as compared with 3R4F cigarette exposure at the air-liquid interface (p < 0.0001). Both THPs demonstrated a statistically similar reduction in biological response, with >87% viability relative to 3R4F at a common aerosol dilution (1:40, aerosol:air). A similar response was observed when plotted against nicotine; a statistical difference between 3R4F and THPs (p < 0.0001) and no difference between the THPs (p = 0.0186). This pre-clinical in vitro biological testing forms part of a larger package of data to help assess the safety and risk reduction potential of next-generation tobacco products relative to cigarettes, using a weight of evidence approach.” (Jaunky et al. 2018).

“Breast cancer (BC) is the most common cancer affecting women worldwide and has been associated with active tobacco smoking. Low levels of nicotine (Nic) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), have been detected in cases of second-hand smoke (SHS). However, the correlation between SHS and BC risk remains controversial. In this study, we investigated whether the physiological SHS achievable dose of Nic and tobacco specific nitrosamine, NNK act together to induce breast carcinogenesis using an in vitro breast cell carcinogenesis model. Immortalized non-tumorigenic breast epithelial cell line, HBL-100 used for a time-course assay, was exposed to very low levels of either Nic or NNK, or both. The time-course assay consisted of 23 cycles of nitrosamines treatment. In each cycle, HBL-100 cells were exposed to 1pM of Nic and/or 100 femtM of NNK for 48 hours. Cells were passaged every 3 days and harvested after 10, 15, and 23 cycles. Our results demonstrated that the tumorigenicity of HBL-100, defined by soft agar colony forming, proliferation, migration and invasion abilities, was enhanced by co-exposure to physiologically SHS achievable doses of Nic and NNK. In addition, α9-nAChR signaling activation, which plays an important role in cellular proliferation and cell survival, was also observed. Importantly, an increase in stemness properties including the prevalence of CD44+/CD24- cells, increase Nanog expression and mammosphere-forming ability were also observed. Our results indicate that chronic and long term exposure to environmental tobacco smoke, may induce breast cell carcinogenesis even at extremely low doses.” (Fararjeh et al. 2019).

“We have previously shown that the chromogranin A (CgA)-derived peptide catestatin (CST: hCgA352-372) inhibits nicotine-induced secretion of catecholamines from the adrenal medulla and chromaffin cells. In the present study, we seek to determine whether CST regulates dense core (DC) vesicle (DCV) quanta (catecholamine and chromogranin/secretogranin proteins) during acute (0.5-h treatment) or chronic (24-h treatment) cholinergic (nicotine) or peptidergic (PACAP, pituitary adenylyl cyclase activating polypeptide) stimulation of PC12 cells. In acute experiments, we found that both nicotine (60 μM) and PACAP (0.1 μM) decreased intracellular norepinephrine (NE) content and increased 3H-NE secretion, with both effects markedly inhibited by co-treatment with CST (2 μM). In chronic experiments, we found that nicotine and PACAP both reduced DCV and DC diameters and that this effect was likewise prevented by CST. Nicotine or CST alone increased expression of CgA protein and together elicited an additional increase in CgA protein, implying that nicotine and CST utilize separate signaling pathways to activate CgA expression. In contrast, PACAP increased expression of CgB and SgII proteins, with a further potentiation by CST. CST augmented the expression of tyrosine hydroxylase (TH) but did not increase intracellular NE levels, presumably due to its inability to cause post-translational activation of TH through serine phosphorylation. Co-treatment of CST with nicotine or PACAP increased quantal size, plausibly due to increased synthesis of CgA, CgB and SgII by CST. We conclude that CST regulates DCV quanta by acutely inhibiting catecholamine secretion and chronically increasing expression of CgA after nicotinic stimulation and CgB and SgII after PACAPergic stimulation.” (Sahu et al. 2018).

“Cigarette smoking has been identified as a major risk factor for osteoporosis decades ago. Several studies have shown a direct relationship between cigarette smoking, decreased bone mineral density, and impaired fracture healing. However, the mechanisms behind impaired fracture healing and cigarette smoking are yet to be elucidated. Migration and osteogenesis of mesenchymal stem/stromal cells (MSCs) into the fracture site play a vital role in the process of fracture healing. In human nicotine, the most pharmacologically active and major addictive component present in tobacco gets rapidly metabolized to the more stable cotinine. This study demonstrates that physiological concentrations of both nicotine and cotinine do not affect the osteogenic differentiation of MSCs. However, cigarette smoke exposure induces oxidative stress by increasing superoxide radicals and reducing intracellular glutathione in MSCs, negatively affecting osteogenic differentiation. Although, not actively producing reactive oxygen species (ROS) nicotine and cotinine inhibit catalase and glutathione reductase activity, contributing to an accumulation of ROS by cigarette smoke exposure. Coincubation with N-acetylcysteine or L-ascorbate improves impaired osteogenesis caused by cigarette smoke exposure by both activation of nuclear factor erythroid 2-related factor 2 (Nrf2) signaling and scavenging of ROS, which thus might represent therapeutic targets to support fracture healing in smokers.” (Aspera-Werz et al. 2018).

“The present study was designed to explore the role of transient receptor potential canonical 3 (TRPC3) in nicotine-induced chronic obstructive pulmonary disease (COPD) and its underlying mechanism. In this study, the expression and localization of α5 nicotinic acetylcholine receptor (α5-nAchR) in lung tissues were determined by western blotting and immunohistochemistry. The quantitative real-time PCR (qRT-PCR) analysis was performed to examine the mRNA expression levels of α5-nAchR and TRPC3 in human airway smooth muscle cells (HASMCs). Cell viability was assessed by CCK-8 assay. Proliferation was detected by cell counting and EdU immunofluorescent staining. Fluorescence calcium imaging was carried out to measure cytosolic Ca2+ ([Ca2+]cyt) concentration. The results showed that the α5-nAchR and TRPC3 expressions were significantly up-regulated in lung tissues of COPD smokers. Nicotine promoted HASMC proliferation, which was accompanied by elevated α5-nAchR and TRPC3 expressions, basal [Ca2+]cyt, store-operated calcium entry (SOCE) and the rate of Mn2+ quenching in HASMCs. Further investigation indicated that nicotine-induced Ca2+ response and TRPC3 up-regulation was reversibly blocked by small interfering RNA (siRNA) suppression of α5-nAChR. The knockdown of TRPC3 blunted Ca2+ response and HASMC proliferation induced by nicotine. In conclusion, nicotine-induced HASMC proliferation was mediated by TRPC3-dependent calcium entry via α5-nAchR, which provided a potential target for treatment of COPD.” (Jiang et al. 2019).

“Nicotine contained in cigarette smoke contributes to the onset of several diseases, including osteoporosis, whose emerging pathogenic mechanism is associated with osteoblasts apoptosis. Scanty information is available on the molecular mechanisms of nicotine on osteoblasts apoptosis and, consequently, on an important aspect of the pathogenesis of smokers-related osteoporosis. Glyoxalase 1 (Glo1) is the detoxification enzyme of methylglyoxal (MG), a major precursor of advanced glycation end products (AGEs), potent pro-apoptotic agents. Hydroimidazolone (MG-H1) is the major AGE derived from the spontaneous MG adduction of arginine residues. The aim of this study was to investigate whether, and by means of which mechanism, the antiglycation defence Glo1 was involved in the apoptosis induced by 0.1 and 1µM nicotine in human primary osteoblasts chronically exposed for 11 and 21 days. By using gene overexpression/silencing and scavenging/inhibitory agents, we demonstrated that nicotine induces a significant intracellular accumulation of hydrogen peroxide (H2O2) that, by inhibiting Glo1, drives MG-H1 accumulation/release. MG-H1, in turn, triggers H2O2 overproduction via receptor for AGEs (RAGE) and, in parallel, an apoptotic mitochondrial pathway by inducing Transglutaminase 2 (TG2) downregulation-dependent NF-kB desensitization. Measurements of H2O2, Glo1 and MG-H1 circulating levels in smokers compared with non-smokers or in smokers with osteoporosis compared with those without this bone-related disease supported the results obtained in vitro. Our findings newly pose the antiglycation enzymatic defense Glo1 and MG-H1 among the molecular events involved in nicotine-induced reactive oxygen species-mediated osteoblasts apoptosis, a crucial event in smoker-related osteoporosis, and suggest novel exposure markers in health surveillance programmes related to smokers-associated osteoporosis.” (Marinucci et al. 2018).

“Exposure to nicotine is known to cause adverse effects in many target organs including kidney. Epidemiological studies suggest that nicotine-induced kidney diseases are prevalent worldwide. However, the impact of duration of exposure on the nicotine-induced adverse effects in normal kidney cells and the underlying molecular mechanism is still unclear. Hence, the objective of this study was to evaluate both acute and long-term effects of nicotine in normal human kidney epithelial cells (HK-2). Cells were treated with 1 and 10 µM nicotine for acute and long-term duration. The result of cell viability showed that the acute exposure to 1 µM nicotine has no significant effect on growth. However, the 10 µM nicotine caused significant decrease in the growth of HK-2 cells. The long-term exposure resulted in significantly increased cell growth in both 1 and 10 µM nicotine-treated groups. Analysis of cell cycle and expression of marker genes related to proliferation and apoptosis further confirmed the effects of nicotine. Additionally, the analysis of growth signaling pathway revealed the decreased level of pAKT in cells with acute exposure whereas the increased level of pAKT in long-term nicotine-exposed cells. This suggests that nicotine, through modulating the AKT pathway, controls the duration-dependent effects on the growth of HK-2 cells. In summary, this is the first report showing long-duration exposure to nicotine causes increased proliferation of human kidney epithelial cells through activation of AKT pathway.” (Chang and Singh 2018).

“Cigarette smoke is associated to pathological weakening of bone tissue, being considered an important playmaker in conditions such as osteoporosis and periodontal bone loss. In addition, it is also associated with an increased risk of failure in bone regeneration strategies. The present work aimed to characterize the effects of nicotine on human osteoclastogenesis over a hydroxyapatite substrate. Osteoclast precursors were maintained in the absence or presence of the osteoclastogenesis enhancers M-CSF and RANKL, and were further treated with nicotine levels representative of the concentrations observed in the plasma and saliva of smokers. It was observed that nicotine at low concentrations elicit an increase in osteoclast differentiation, but only in the presence of M-CSF and RANKL it was also able to significantly increase the resorbing ability of osteoclasts. A slight downregulation of NFkB pathway and an increase in the production of TNF-α and, particularly PGE2, were involved in the observed effects of nicotine. At high concentrations, nicotine revealed cytotoxic effects, causing a decrease in cell density. In conclusion, nicotine at levels found in the plasma of the smokers, has the ability to act directly on osteoclast precursors, inducing its osteoclastogenic differentiation. The stimulatory behavior appears to be dependent on the stage of osteoclastic differentiation of the precursor cells, which means, in the absence of M-CSF and RANKL, it only favors the initial stages of osteoclast differentiation, while in the presence of the growth factors, a significant increase in their resorbing ability is also achieved.” (Costa-Rodrigues et al. 2018).

“The diffusion of e-cigarette (e-CIG) opens a great scientific and regulatory debate about its safety. The huge number of commercialized devices, e-liquids with almost infinite chemical formulations and the growing market demand for a rapid and efficient toxicity screen system that is able to test all of these references and related aerosols. A consensus on the best protocols for the e-CIG safety assessment is still far to be achieved, since the huge number of variables characterizing these products (e.g., flavoring type and concentration, nicotine concentration, type of the device, including the battery and the atomizer). This suggests that more experimental evidences are needed to support the regulatory frameworks. The present study aims to contribute in this field by testing the effects of condensed aerosols (CAs) from three main e-liquid categories (tobacco, mint, and cinnamon as food-related flavor), with (18 mg/mL) or without nicotine. Two in vitro models, represented by a monoculture of human epithelial alveolar cells and a three-dimensional (3D) co-culture of alveolar and lung microvascular endothelial cells were used. Cell viability, pro-inflammatory cytokines release and alveolar-blood barrier (ABB) integrity were investigated as inhalation toxicity endpoints. Results showed that nicotine itself had almost no influence on the modulation of the toxicity response, while flavor composition did have. The cell viability was significantly decreased in monoculture and ABB after exposure to the mints and cinnamon CAs. The barrier integrity was significantly affected in the ABB after exposure to cytotoxic CAs. With the exception of the significant IL-8 release in the monoculture after Cinnamon exposure, no increase of inflammatory cytokines (IL-8 and MCP-1) release was observed. These findings point out that multiple assays with different in vitro models are able to discriminate the acute inhalation toxicity of CAs from liquids with different flavors, providing the companies and regulatory bodies with useful tools for the preliminary screening of marketable products.” (Bengalli et al. 2017).

“BACKGROUND/AIMS: The proliferation of human bronchial smooth muscle cells (HBSMCs) is a key pathophysiological component of airway remodeling in chronic obstructive pulmonary disease (COPD) for which pharmacotherapy is limited, and only slight improvements in survival have been achieved in recent decades. Cigarette smoke is a well-recognized risk factor for COPD; however, the pathogenesis of cigarette smoke-induced COPD remains incompletely understood. This study aimed to investigate the mechanisms by which nicotine affects HBSMC proliferation. METHODS: Cell viability was assessed with a CCK-8 assay. Proliferation was measured by cell counting and EdU immunostaining. Fluorescence calcium imaging was performed to measure intracellular Ca2+ concentration ([Ca2+]i). RESULTS: The results showed that nicotine promotes HBSMC proliferation, which is accompanied by elevated store-operated calcium entry (SOCE), receptor-operated calcium entry (ROCE) and basal [Ca2+]i in HBSMCs. Moreover, we also confirmed that canonical transient receptor potential protein 6 (TRPC6) and α7 nicotinic acetylcholine receptor (α7 nAChR) are involved in nicotine-induced upregulation of cell proliferation. Furthermore, we verified that activation of the PI3K/Akt signaling pathway plays a pivotal role in nicotine-enhanced proliferation and calcium influx in HBSMCs. Inhibition of α7 nAChR significantly decreased Akt phosphorylation levels, and LY294002 inhibited the protein expression levels of TRPC6. CONCLUSION: Herein, these data provide compelling evidence that calcium entry via the α7 nAChR-PI3K/Akt-TRPC6 signaling pathway plays an important role in the physiological regulation of airway smooth muscle cell proliferation, representing an important target for augmenting airway remodeling.” (Hong et al. 2017).

## *5.6.* *Carcinogenicity*

“Store‑operated calcium entry (SOCE) is critical for regulating the proliferation and metastasis of various cancer types. The present study aimed to investigate the role of SOCE on nicotine‑promoted proliferation of non‑small cell lung cancer (NSCLC) A549 cells. Cell proliferation was evaluated by BrdU incorporation assay. The SOCE and basal [Ca2+]i in NSCLC A549 cells were determined using Fura‑2 fluorescence microscopy. The mRNA and protein expression levels were determined by real‑time quantitative PCR and western blotting, respectively. The results demonstrated that, in A549 cells, the detectable store‑operated calcium channel (SOCC) components were TRPC proteins 1, 3, 4 and 6 and Orail, among which TRPC1, TRPC6 and Orai1 are expressed at relatively high levels with TRPC3 and TRPC4 at relatively low levels. Nicotine upregulated the mRNA and protein expression of TRPC1, TRPC6 and Orai1, increased basal [Ca2+]i and enhanced SOCE. Promotion of cell proliferation but not migration was observed in the nicotine‑treated cells, which was inhibited by SOCE inhibitor SKF‑96365. Furthermore, nicotine upregulated HIF‑1α expression in the A549 and NCI‑H292 cells. Silencing of HIF‑1α abrogated the increases in TRPCs and Orail and reversed the increases in basal [Ca2+]i and SOCE. Meanwhile, suppression of proliferation was observed in cells following HIF‑1α silencing. In conclusion, the results indicate that nicotine promotes lung cancer cell proliferation likely by upregulating HIF‑1α and SOCC components and therefore enhancing SOCE and increasing basal [Ca2+]i.” (Wang Y et al. 2018a).

“BACKGROUND AND PURPOSE: Tobacco smoke contains many classes of carcinogens and although nicotine is unable to initiate tumourigenesis in humans and rodents, it promotes tumour growth and metastasis in lung tumours by acting on neuronal nicotinic ACh receptors (nAChRs). The aim of this study was to identify molecularly, biochemically and pharmacologically which nAChR subtypes are expressed and functionally activated by nicotine in lung cancer cell lines. EXPERIMENTAL APPROACH: We used A549 and H1975 adenocarcinoma cell lines derived from lung tumours to test the in vitro effects of nicotine, and nAChR subtype-specific peptides and compounds. KEY RESULTS: The two adenocarcinoma cell lines express distinctive nAChR subtypes, and this affects their nicotine-induced proliferation. In A549 cells, nAChRs containing the α7 or α9 subunits not only regulate nicotine-induced cell proliferation but also the activation of the Akt and ERK pathways. Blocking these nAChRs by means of subtype-specific peptides, or silencing their expression by means of subunit-specific siRNAs, abolishes nicotine-induced proliferation and signalling. Moreover, we found that the α7 antagonist MG624 also acts on α9-α10 nAChRs, blocks the effects of nicotine on A549 cells and has dose-dependent cytotoxic activity. CONCLUSIONS AND IMPLICATIONS: These results highlight the pathophysiological role of α7- and α9-containing receptors in promoting non-small cell lung carcinoma cell growth and intracellular signalling and provide a framework for the development of new drugs that specifically target the receptors expressed in lung tumours.” (Mucchietto et al. 2018).

“The tumor microenvironment plays an important role in tumor initiation and progression. It is well documented that nicotine participates in cigarette smoking-related malignancies. Previous studies focused on the effects of nicotine on tumor cells; however, the role of the microenvironment in nicotine-mediated tumorigenesis is poorly understood. Herein, we investigated the effect and molecular mechanism of nicotine on fibroblasts and its contribution to breast cancer. We found that nicotine induced the epithelial-mesenchymal transition (EMT) of breast cancer cells and promoted activation of fibroblasts. Interestingly, conditioned medium from nicotine-activated fibroblasts (Nic-CM) had a greater impact on promoting the EMT and migratory capability toward cancer cells than did treatment with nicotine alone. Production of connective tissue growth factor (CTGF) and transforming growth factor (TGF)-β by nicotine-treated fibroblasts was demonstrated to be crucial for promoting the EMT and cancer cell migration, and blocking of CTGF and TGF-β in Nic-CM-suppressed tumor motility. Moreover, nicotine induced expressions of CTGF, and TGF-β in fibroblasts as identified through α7 nicotinic acetylcholine receptor (nAChR)-dependent activation of the AKT/TAZ signaling mechanism. Together, our data showed for the first time that activation of fibroblasts is largely responsible for accelerating smoking-mediated breast cancer progression.” (Chen P et al. 2018).

“Cigarette smoking is a recognized risk factor for colon cancer and nicotine, the principal active component of tobacco, plays a pivotal role in increasing colon cancer cell growth and survival. The aim of this study was to determine the effect of nicotine on cellular Caco-2 and HCT-8 migration and invasion, focusing on epithelial to mesenchymal transition (EMT) induction, and COX-2 pathway involvement. In both these cell lines, treatment with nicotine increased COX-2 expression and the release of its enzymatic product PGE2 . Moreover, nicotine-stimulated cells showed increased migratory and invasive behavior, mesenchymal markers up-regulation and epithelial markers down-regulation, nuclear translocation of the β-catenin, increase of MMP-2 and MMP-9 activity, and enhanced NF-κB expression. Noticeably, all these effects are largely mediated by COX-2 activity, as simultaneous treatment of both cell lines with nicotine and NS-398, a selective COX-2 inhibitor, greatly reduced the number of migrating and invading cells and reverted nicotine-induced EMT. These findings emphasize that nicotine triggers EMT, leading hence to increased migration and invasiveness of colon cancer cells. Thereby, the use of COX-2 inhibitor drugs might likely counteract nicotine-mediated EMT effects on colon cancer development and progression.” (Dinicola et al. 2018).

“Cytochrome P450 2A13 (CYP2A13) is responsible for the metabolism of chemical compounds such as nicotine, coumarin, and tobacco-specific nitrosamine. Several of these compounds have been recognized as procarcinogens activated by CYP2A13. We recently showed that CYP2A13\*2 contributes to inter-individual variations observed in bladder cancer susceptibility because CYP2A13\*2 might cause a decrease in enzymatic activity. Other CYP2A13 allelic variants may also affect cancer susceptibility. In this study, we performed an in vitro analysis of the wild-type enzyme (CYP2A13.1) and 8 CYP2A13 allelic variants, using nicotine and coumarin as representative CYP2A13 substrates. These CYP2A13 variant proteins were heterologously expressed in 293FT cells, and the kinetic parameters of nicotine C-oxidation and coumarin 7-hydroxylation were estimated. The quantities of CYP2A13 holoenzymes in microsomal fractions extracted from 293FT cells were determined by measuring reduced carbon monoxide-difference spectra. The kinetic parameters for CYP2A13.3, CYP2A13.4, and CYP2A13.10 could not be determined because of low metabolite concentrations. Five other CYP2A13 variants (CYP2A13.2, CYP2A13.5, CYP2A13.6, CYP2A13.8, and CYP2A13.9) showed markedly reduced enzymatic activity toward both substrates. These findings provide insights into the mechanism underlying inter-individual differences observed in genotoxicity and cancer susceptibility.” (Kumondai et al. 2018).

“Breast cancer (BC) is the most common cancer affecting women worldwide and has been associated with active tobacco smoking. Low levels of nicotine (Nic) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), have been detected in cases of second-hand smoke (SHS). However, the correlation between SHS and BC risk remains controversial. In this study, we investigated whether the physiological SHS achievable dose of Nic and tobacco specific nitrosamine, NNK act together to induce breast carcinogenesis using an in vitro breast cell carcinogenesis model. Immortalized non-tumorigenic breast epithelial cell line, HBL-100 used for a time-course assay, was exposed to very low levels of either Nic or NNK, or both. The time-course assay consisted of 23 cycles of nitrosamines treatment. In each cycle, HBL-100 cells were exposed to 1pM of Nic and/or 100 femtM of NNK for 48 hours. Cells were passaged every 3 days and harvested after 10, 15, and 23 cycles. Our results demonstrated that the tumorigenicity of HBL-100, defined by soft agar colony forming, proliferation, migration and invasion abilities, was enhanced by co-exposure to physiologically SHS achievable doses of Nic and NNK. In addition, α9-nAChR signaling activation, which plays an important role in cellular proliferation and cell survival, was also observed. Importantly, an increase in stemness properties including the prevalence of CD44+/CD24- cells, increase Nanog expression and mammosphere-forming ability were also observed. Our results indicate that chronic and long term exposure to environmental tobacco smoke, may induce breast cell carcinogenesis even at extremely low doses.” (Fararjeh et al. 2019).

“Epidermal growth factor (EGF) is overexpressed in many cancers and is associated with worse prognosis. EGF binds to its cell surface receptor (EGFR), which induces EGFR phosphorylation. Phosphorylated EGFR (p‑EGFR) is translocated into the nucleus, which increases cancer cell activity. Nicotine, which is one of the main components of tobacco, is absorbed through pulmonary alveoli and mucosal epithelia in the head and neck region by smoking and moves into the blood. Nicotine in blood binds to nicotinic acetylcholine receptor (nAChR) in the central nervous system and serves a crucial role in tobacco addiction. Although nAChR localization is thought to be limited in the nervous system, nAChR is present in a wide variety of non‑neuronal cells, including cancer cells. Recent studies suggest that nicotine contributes to the metastasis and resistance to anti‑cancer drugs of various cancer cells. However, it remains unknown whether head and neck squamous cell carcinoma (HNSCC) cells can utilize nicotine‑nAChR signaling to metastasize and acquire resistance to anti‑cancer drugs, even though the mucosal epithelia of the head and neck region are the primary sites of exposure to tobacco smoke. To the best of our knowledge, the present study is the first to demonstrate the role of nicotine in metastasis and anti‑EGFR‑therapy resistance of HNSCC. The present findings demonstrated that nicotine increased proliferation, migration, invasion, p‑EGFR nuclear translocation and protein kinase B (Akt) phosphorylation in HNSCC cells. It was also demonstrated that nicotine restored cetuximab‑inhibited proliferation, migration and invasion of HNSCC cells. Finally, an in vivo experiment revealed that nicotine increased lymph node metastasis of xenografted tumors, whereas an nAChR inhibitor suppressed lymph node metastasis and p‑EGFR nuclear localization of xenografted tumors. Taken together, these results demonstrated that nicotine induced nuclear accumulation of p‑EGFR, and activation of Akt signaling. These signaling pathways elevated the activities of HNSCC cells, causing lymph node metastasis and serving a role in cetuximab resistance.” (Shimizu et al. 2019).

“Recent studies show substantial growth-promoting properties of nicotine (NIC) in cancer, which is a combined outcome of genetic and epigenetic alterations. However, the role of epigenetic modifiers in response to NIC in breast cancer is less studied. In the present study, for the first time we have shown NIC-induced enhanced EZH2 expression. Six pairs of smoking-associated breast cancer patient tissues were analyzed. Samples from smoking breast cancer patients showed distinguished enhanced EZH2 expression in comparison to non-smoking ones. The upregulation in EZH2, which is due to NIC, was further confirmed in breast carcinoma cell lines using 10 µM NIC, 1 µM DZNepA, and EZH2si. The upregulation of EZH2 was concomitant with upregulation in Myc and α9-nAChR. The xenograft of breast cancer cells in BALB/c nude mice in the presence or absence of NIC showed significantly higher tumor uptake in the NIC injected group, which clearly demonstrates the effect of NIC in breast cancer progression. Interestingly, DZNepA considerably suppressed the NIC-mediated tumor growth. CHIP-qPCR assay confirmed the increased Myc enrichment on EZH2 promoter upon NIC treatment, thereby strengthening our findings that there exists an association between NIC, Myc, and EZH2. Overall, the present study identifies a strong association between NIC and EZH2 particularly in the progression of breast cancer in smokers through a novel axis involving nAChR and Myc. Moreover, the findings provide preliminary evidence suggesting potential of high level of EZH2 expression as a prognostic marker in smoking-associated breast cancer.” (Kumari et al. 2018).

“Tobacco smoking is a major risk factor for human cancers including urinary bladder carcinoma. Cigarette smoke inhalation in mice and orally administered nicotine in rats and mice increased urothelial cell proliferation. Nicotine, a major component of smoke, induced cell proliferation in multiple cell types in vitro. In the present study, the enhancing effects of nicotine on F344 rat bladder carcinogenesis induced by N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) were examined. Nicotine administered in drinking water for 32 weeks following 4 weeks of BBN treatment significantly increased the incidence and number of urothelial carcinomas dose-dependently. Ki67 and pSTAT3 labeling indices and expression of nicotinic acetylcholine receptor alpha 7 (nAChRα7) in non-tumor bladder urothelial lesions were significantly increased by nicotine, but the TUNEL assay for apoptosis showed no increase. In a 4 week study, inhibitors of nicotinic acetylcholine receptor decreased nicotine-induced urothelial simple hyperplasia and Ki67 labeling index in the bladder and kidney pelvis at a single cytotoxic dose of nicotine (40 ppm). Urothelial cytotoxicity with regenerative proliferation was observed by light and scanning electron microscopy. In vitro, nicotine was not cytotoxic to rat or human immortalized urothelial cells (do not express nicotine receptors) below millimolar concentrations, nor in human RT4, T24 or UMUC3 urothelial carcinoma cells (express nicotine receptors). However, nicotine slightly, but statistically significantly, increased cell proliferation at micromolar concentrations in human urothelial carcinoma cells. These data suggest that nicotine enhances urinary bladder carcinogenesis by inducing cytotoxicity with regenerative proliferation. The possible role of direct mitogenesis, involving nAChR and STAT3 signaling and of nicotine receptors requires further investigation at non-cytotoxic doses of nicotine.” (Suzuki et al. 2018).

“BACKGROUND: Tobacco smoke is by far the greatest risk factor for non-small-cell lung cancer (NSCLC). Nicotine, an active alkaloid in tobacco, is unable to initiate tumorigenesis in humans and rodents, but can promote the growth and metastasis of various tumors, including NSCLC, initiated by tobacco carcinogens. Recently, cigarette smoke is reported to downregulate 24 miRNAs more than 3-fold in the lungs of rats, and most of these downregulated miRNAs are associated with NSCLC initiation and development. Nicotine as the major tobacco component might be associated with the expression changes of some miRNAs. METHODS: qRT-PCR was performed to determine the miRNA and mRNA expression, and western blot was conducted to measure protein expression. MTT assay was used to detect cell proliferation. RESULTS: The effects of nicotine on the expression of 24 miRNAs in NSCLC cell lines were determined, and the results showed that nicotine treatment decreased miR-99b and miR-192 expression. Cell proliferation and epithelial-to-mesenchymal transition (EMT) detection showed that nicotine promoted NSCLC cell proliferation and EMT, and restoration of miR-99b or miR-192 expression relieved the effects of nicotine on NSCLC cell proliferation and EMT. Subsequently, fibroblast growth factor receptor 3 (FGFR3) and retinoblastoma 1 (RB1) were confirmed to be the targets of miR-99b and miR-192, respectively, and were upregulated by nicotine in NSCLC cells. In addition, FGFR3 or RB1 knockdown inhibited NSCLC cell proliferation and EMT. CONCLUSION: This study, for the first time, elucidates nicotine-miR-99b/miR-192-FGFR3/RB1 regulatory network that nicotine promotes NSCLC cell proliferation and EMT by downregulating miR-99b and miR-192, and upregulating their targets FGFR3 and RB1. These findings offer novel insights into the understanding of underlying molecular mechanisms of NSCLC related with the nicotine effects.” (Du et al. 2018).

“Tobacco smoking is an independent risk factor for the initiation of pancreatic cancer (PC). Hypermethylation of tumor suppressor genes has been demonstrated to be associated with smoking. This study aimed to find the relationship between nicotine exposure and hypermethylation of tumor suppressor genes in normal pancreatic epithelial cells. Human pancreatic epithelial cells ware cultured exposing to nicotine and the methylation status of tumor suppressor genes were detected. Proenkephalin (PENK) was chosen as the target gene and methylation level of PENK promoter region was measured. Expression of DNA methyltransferase (DNMT), nicotine acetylcholine receptor (α7nAChR) and signaling pathway downstream were analyzed. Nicotine induces overexpression of DNMT3A and 3B, and methylated-inactivation of PENK gene in normal pancreatic epithelial cells. An activation of α7nAChR and MAPK signaling pathway has been detected in the nicotine-treated group. Demethylated drug, antagonist of α7nAChR and inhibitor of p38 MAPK is verified to attenuate the overexpression of DNMTs stimulated by nicotine as well as inhibit aberrant hypermethylation-related silence of PENK gene. Nicotine stimulation can induce aberrant hypermethylation of tumor suppressor genes by α7nAChR and MAPK signaling pathway-mediated up-regulation of DNMTs in pancreatic epithelial cells, thus we can provide epigenetic evidence of the mechanisms by which smoking causes pancreatic cancer and find new therapeutic target.” (Jin et al. 2018).

“Studies in animal models have suggested that nicotine, an agonist of nicotinic acetylcholine receptors, may have the potential to prevent and/or reverse the peripheral neuropathy induced by cancer chemotherapeutic drugs, such as paclitaxel and oxaliplatin. However, a large body of evidence suggests that nicotine may also stimulate lung tumor growth and/or interfere with the effectiveness of cancer chemotherapy. Whereas the reported proliferative effects of nicotine are highly variable, the antagonism of antitumor drug efficacy is more consistent, although this latter effect has been demonstrated primarily in cell culture studies. In contrast, in vitro and in vivo studies from our own laboratory indicate that nicotine fails to enhance the growth of nonsmall cell lung cancer cells or attenuate the effects of chemotherapy (paclitaxel). Given the inconsistencies in the literature, coupled with our own findings, the weight of evidence suggests that caution may be warranted in proposing to use nicotine to mitigate chemotherapy-induced peripheral neuropathy in cancer patients receiving chemotherapy. Conversely, clinical trials could be performed in patients who have completed therapy and are considered to be disease-free to determine whether nicotine, in the form of commercially available patches or gum, is effective in alleviating peripheral neuropathy symptoms.” (Kyte and Gewirtz 2018).

There is some biological basis indicating that nicotine may promote cancer based on experimental studies that have limitations in replicating human exposure and on mechanistic studies (Chen et al. 2008; Chen et al. 2010; Catassi et al. 2008). Also, there is evidence that nicotine may promote metastases by stimulating cell motility and migration, loss of adhesion and inducing the transition the transition of a well-differentiated epithelial cell to a highly invasive carcinoma via mesenchymal transition (Catassi et al. 2008; Eglenton et al., 2008). However, very little data are on human cancer risk relating to nicotine.

The Lung Health Study provides information about long-term users of NRT (nicotine replacement therapy) (Murry et al., 2009). Even though the study was not designed to directly examine nicotine’s potential cancer risk. It was a 5-year randomized trial to assess the effects of smoking cessation and reduction on chronic lung disease and lung function. Among 5887 subjeczs initially enrolled, the researchers continued to follow some of them for additional 7.5 years (n=3220). Study participants were offered NRT (Nicorette gum) without consideration of randomization or study design. Although they were encouraged to use NRT for only 6 months, many continued to use it long term. A total of 75 lung cancers were diagnosed among smokers and quitters of the extended surveillance group, but the use of NRT was not associated with lung cancer (or other cancers). A major limitation was the short follow-up period of only 7 additional years. Notwithstanding the limitations, this study at least did not indicate a strong role for nicotine in promoting carcinogenesis in humans.

“In inhalation study in female Sprague-Dawley rats (n=68), animals exposed to about 0.5 mg/m3 of nicotine, for 20 hours per day, 5 days per week, for 103 weeks. Non-exposed rats served as a control group. Mean nicotine concentrations in plasma measured after 5 days and at the end of the study, were 108 µg/L and 130 µg/L. No increase in mortality, atherosclerosis and frequency of tumors were observed in treated rats compared with control animals. Additionally, there was no microscopic or macroscopic lung tumors nor any increase in pulmonary neuroendocrine cells. Examination of other tissues including brain, gastrointestinal tract, liver and kidneys did not reveal treatment-related abnormalities. Throughout the study, however, the body weight of the nicotine exposed rats was reduced as compared with control rats.” (Waldum et al. 1996).

Murphy et al. (2011) determined the effect of nicotine administration on 4- (methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumors in A/J mice (a species which is highly susceptible to lung tumors). Female mice were administered a single dose of NNK (10 μmol) and 0.44 μmol/ml nicotine in the drinking water. Nicotine was administered 2 weeks prior to NNK, 44 weeks after NNK, throughout the experiment, or without NNK treatment. In summary, there were three key outcomes from the studies on the effect of nicotine on tumorigenesis in the A/J mouse: 1) Chronic nicotine consumption alone did not induce lung tumors. 2) Nicotine consumption up until the time of NNK administration did not decrease (or increase) tumor incidence and 3) chronic nicotine consumption had no significant effect on tumor incidence, multiplicity or progression.

“For harms of longer term use of nicotine, the best evidence stems from snus, described earlier. The Global Burden of Disease Study (94), did not find sufficient evidence of a detrimental effect of snus on any outcome. This includes oral and pharyngeal cancer which had both been linked with smokeless tobacco use in general, and the latter with snus.

The best study of long-term NRT use, dates from 2009, the Lung Health study (93), a randomised controlled trial of five years duration, in which all subjects were offered NRT and subjects were followed up for up to seven and a half years. There was no evidence of a relationship between NRT and cancers, whereas continued smoking was associated with developing cancer.“ (Public Health England, 2018)

“Cluster of differentiation 24 (CD24) is a widely used cancer stem cell (CSC) marker in numerous cancer types. However, a number of studies have shown that CD24 is a prognostic marker, but not a CSC marker for lung adenocarcinoma. In the present study, firstly, bioinformatic analyses were used to identify the CD24 mRNA levels in the subtypes of lung cancer. Secondly, CD24high and CD24low cells were isolated from the side population of Lewis lung carcinoma (LLC) cells using flow cytometry. Furthermore, the stemness of CD24high and CD24low cells were determined in vivo and in vitro. Lastly, the mechanism(s) of nicotine-inhibited CD24 expression in LLC cells were assessed. The main findings of this study are that: i) CD24 could be used as a prognostic marker for human lung adenocarcinoma; ii) the in vitro and in vivo experiments did not determine a significant influence of CD24 on the tumorgenicity of LLC cells; and iii) nicotine inhibited CD24 expression in LLC cells by upregulation of RAS. However, the detailed mechanism(s) of these results require further analysis.” (Liu D et al. 2018).

“Tobacco smoking is an independent risk factor for the initiation of pancreatic cancer (PC). Hypermethylation of tumor suppressor genes has been demonstrated to be associated with smoking. This study aimed to find the relationship between nicotine exposure and hypermethylation of tumor suppressor genes in normal pancreatic epithelial cells. Human pancreatic epithelial cells ware cultured exposing to nicotine and the methylation status of tumor suppressor genes were detected. Proenkephalin (PENK) was chosen as the target gene and methylation level of PENK promoter region was measured. Expression of DNA methyltransferase (DNMT), nicotine acetylcholine receptor (α7nAChR) and signaling pathway downstream were analyzed. Nicotine induces overexpression of DNMT3A and 3B, and methylated-inactivation of PENK gene in normal pancreatic epithelial cells. An activation of α7nAChR and MAPK signaling pathway has been detected in the nicotine-treated group. Demethylated drug, antagonist of α7nAChR and inhibitor of p38 MAPK is verified to attenuate the overexpression of DNMTs stimulated by nicotine as well as inhibit aberrant hypermethylation-related silence of PENK gene. Nicotine stimulation can induce aberrant hypermethylation of tumor suppressor genes by α7nAChR and MAPK signaling pathway-mediated up-regulation of DNMTs in pancreatic epithelial cells, thus we can provide epigenetic evidence of the mechanisms by which smoking causes pancreatic cancer and find new therapeutic target.” (Jin et al. 2018).

“Nicotine, a tumor promoter in tobacco, can increase Peroxiredoxin (Prx1) and nicotinic acetylcholine receptors (nAChRs) in oral squamous cell carcinoma (OSCC). In the present study, we investigate the effects of nicotine in oral precancerous lesions focusing on apoptosis and nAChR/Prx1 signaling. We detected expression of Prx1, α3nAChR, α7nAChR, phosphorylation of mitogen-activated protein kinases (MAPK) and apoptosis in dysplastic oral keratinocyte (DOK) cells as well as in 4-nitroquinoline 1-oxide (4NQO) or 4NQO + nicotine - induced oral precancerous lesions in Prx1 wild-type (Prx1+/+) and Prx1 knockdown (Prx1+/-) mice. In DOK cells, Prx1 knockdown and blocking α7nAChR activated apoptosis, and nicotine increased the expression of Prx1, α3nAChR and α7nAChR, and inhibited MAPK activation. Moreover, nicotine suppressed apoptosis depending on Prx1 and α7nAChR in DOK cells. In animal bioassay, nicotine and Prx1 promoted growth of 4NQO-induced precancerous lesions in mouse tongue. 4NQO plus nicotine suppressed MAPK activation in Prx1 wild-type mice but not in Prx1 knockdown mice. Our data demonstrate that nicotine inhibits cell apoptosis and promotes the growth of oral precancerous lesions via regulating α7nAChR/Prx1 during carcinogenesis of OSCC.” (Wang et al. 2017).

## *5.7.* *Irritation/immunotoxicity*

“Cigarette smoke has significant toxic effects on the immune system, and increases the risk of developing autoimmune diseases; one immunosuppressive effect of cigarette smoke is that it inhibits the T cell-stimulating, immunogenic properties of myeloid dendritic cells (DCs). As the functions of DCs are regulated by intra-cellular signaling pathways, we investigated the effects of cigarette smoke extract (CSE) and nicotine on multiple signaling molecules and other regulatory proteins in human DCs to elucidate the molecular basis of the inhibition of DC maturation and function by CSE and nicotine. Maturation of monocyte-derived DCs was induced with the TLR3-agonist poly I:C or with the TLR4-agonist lipopolysaccharide, in the absence or presence of CSE or nicotine. Reverse-phase protein microarray was used to quantify multiple signaling molecules and other proteins in cell lysates. Particularly in poly I:C-matured DCs, cigarette smoke constituents and nicotine suppressed the expression of signaling molecules associated with DC maturation and T cell stimulation, cell survival and cell migration. In conclusion, constituents of tobacco smoke suppress the immunogenic potential of DCs at the signaling pathway level.” (Alkhattabi et al. 2018).

“Although the risks of smoking are well known, the effects of exposure to nicotine on endocrine functions remain unclear. We investigated the deleterious effects of nicotine on the adrenal gland and the mechanisms of these changes in rats. The role of melatonin in ameliorating pathological changes also was investigated. We used 24 rats divided into four groups of six: group 1, control; group 2, nicotine treated; group 3, nicotine and melatonin treated; group 4, melatonin treated. We used histology; immunohistochemistry of inducible nitric oxide synthase (iNOS), vascular endothelial growth factor (VEGF) and tyrosine hydroxylase (TH); measured oxidative and antioxidative markers, malondialdehyde (MDA) and glutathione (GSH); and performed real-time PCR for NF-κB 65, IL1-B and IL6. We also performed histomorphometric analysis. Indentation and lamellar separation of the adrenal capsule, vacuolated degenerated cells and lymphocytic infiltration were observed in group 2. Vacuolated cells and cells with pyknotic nuclei also were detected in the zona reticularis and medulla of the same group. We observed improved shape and cellular lining of the gland in groups 3 and 4. Widespread expression of iNOS, VEGF and TH, increased area percent collagen, decreased GSH (56%) and increased MDA, NF-κB, IL1-B and IL-6 were observed in group 2. All parameters were ameliorated in groups 3 and 4. The effects of nicotine on the adrenal gland can be attributed to oxidative and inflammatory stress; melatonin ameliorates these effects.” (Abdel Fattah et al. 2019).

“PURPOSE: To build a murine model for tobacco smoke and electronic cigarette vapor exposure to characterize the inflammatory and immune responses in the larynx. MATERIALS AND METHODS: In this pilot study, twenty-four wild-type C57BL/6 mice were divided into four groups: smoke, vapor with nicotine, vapor without nicotine, and air only. Following daily exposure for 4 months, larynges were dissected and processed with cytokine detection arrays. Each laryngeal cytokine level between the four different groups was analyzed statistically by using statistical analysis software (SAS) to calculate the analysis of variance (ANOVA). RESULTS: IL-4 was the only cytokine found to achieve statistically significant different levels in this study, with elevated levels of IL-4 in the tobacco smoke and vapor with nicotine groups compared to the levels found in the vapor without nicotine and air only groups (p = 0.0418). While statistically non-significant, prominent findings revealed up-regulation of TGF-β2 and TGF-β3 in the smoke group, but near-normal levels of TGF-β2 and TGF-β3 and suppression of IL-10 in the vapor groups (p > 0.05). CONCLUSION: The potential utility of the murine model is established for studying the inflammatory and immune effects of tobacco smoke and vapor on the mammalian larynx. IL-4 levels in mice larynges were significantly elevated in the tobacco smoke and vapor with nicotine groups.” (Ha et al. 2019).

Several studies reporting local skin reactions to nicotine and nicotine patches were identified.

In an evaluation of 1392 patients undergoing transdermal nicotine therapy for smoking, Gourlay et al. (1991) reported erythema in 14.7%, rash in 5.2% puritis in 20.8%, irritation in 4.7%, vesicles in 4.9% and edema in 3.8% of patients. However, study participants with pre-existing skin disorders were more likely to report mild application site reactions than other participants.

“In the meta-analysis from 47 reports of 35 clinical trials (total of 9253 patients including 5501 in the nicotine-patch group and 3752 placebo receiving patients) localized skin irritation was reported as one of the several minor adverse effects affecting 25% of patients in the nicotine-patch groups.” (Greenland et al. 1998).

“Nicotine is not particularly antigenic, and nicotine allergy in smokers and tobacco workers is rare. However, allergic contact dermatitis from nicotine has been reported among nicotine TTS users. It is hypothesized that cutaneous exposure to nicotine allows the molecule to penetrate the epidermis, and binding of this hapten with skin proteins may form a neoantigen, which may induce sensitization.” (Bircher et al. 1991).

“Sensitization responses to pure nicotine were studied in 10 males and 4 females, who had previously experienced adverse skin reactions from the use of nicotine patches. Tests were conducted with aqueous solutions of 1%, 10%, and 50% nicotine base and 5% nicotine sulphate, applied under occlusion to the backs of the subjects for 2 to 3 days. The incidences of positive allergic patch test reactions to nicotine base were 1/14, 4/14, and 5/14 at concentrations of 1%, 10%, or 50%, respectively, and 1/14 when exposed to nicotine sulphate (5%). At the high concentration, irritant reactions due to occlusion were present in the remaining 9 subjects.” (Bircher et al. 1991).

Several other studies have reported cutaneous reactions to nicotine. In a study involving 183 smokers, during the treatment period, 53% of the patients complained of pruritus and 39% developed erythemas that were almost exclusively confined to those areas where the patches had been applied. However, most symptoms appeared only in slight or moderate forms. In contrast to irritative cutireactions, 6 patients developed a genuine contact allergy (type IV, delayed type reaction) which proved in 5 cases to be induced by the nicotine contained in the patches (Eichelberg et al. 1989).

No data found on skin irritation or skin sensitization properties of nicotine in animals.

## *5.8.* *All other relevant types of toxicity*

“Cigarette smoke is associated to pathological weakening of bone tissue, being considered an important playmaker in conditions such as osteoporosis and periodontal bone loss. In addition, it is also associated with an increased risk of failure in bone regeneration strategies. The present work aimed to characterize the effects of nicotine on human osteoclastogenesis over a hydroxyapatite substrate. Osteoclast precursors were maintained in the absence or presence of the osteoclastogenesis enhancers M-CSF and RANKL, and were further treated with nicotine levels representative of the concentrations observed in the plasma and saliva of smokers. It was observed that nicotine at low concentrations elicit an increase in osteoclast differentiation, but only in the presence of M-CSF and RANKL it was also able to significantly increase the resorbing ability of osteoclasts. A slight downregulation of NFkB pathway and an increase in the production of TNF-α and, particularly PGE2, were involved in the observed effects of nicotine. At high concentrations, nicotine revealed cytotoxic effects, causing a decrease in cell density. In conclusion, nicotine at levels found in the plasma of the smokers, has the ability to act directly on osteoclast precursors, inducing its osteoclastogenic differentiation. The stimulatory behavior appears to be dependent on the stage of osteoclastic differentiation of the precursor cells, which means, in the absence of M-CSF and RANKL, it only favors the initial stages of osteoclast differentiation, while in the presence of the growth factors, a significant increase in their resorbing ability is also achieved.” (Costa-Rodrigues et al. 2018).

“The present study investigated the influence of cigarette smoke extract (CSE) and nicotine on the expression of thrombomodulin (TM) and endothelial protein C receptor (EPCR) in human umbilical vein endothelial cells (HUVECs). Smoking is associated with intravascular thrombosis. As a vital anticoagulation cofactor, TM is located on the endothelial cell surface and regulates intravascular coagulation by binding to thrombin, hence activating protein C. Activated protein C is a natural anticoagulant that interacts with EPCR to enhance the function of anticoagulation system. The effects of CSE (0.5‑5%) and nicotine (10‑3‑10‑9 mol/l) on the expression of TM and EPCR in HUVECs were observed. Reverse transcription‑quantitative polymerase chain reaction and flow cytometric analysis techniques were used for detecting TM and EPCR mRNA and protein expression levels, respectively. After 6‑h exposure, TM protein and mRNA expression levels decreased in a dose‑dependent manner. Stimulation with 5% CSE for 0, 6, 10, 12 and 24 h led to a decrease in the levels of TM mRNA and protein over time, which reached a peak at 12 h. The levels were significantly reduced compared with the control group (P<0.001). However, CSE had no effect on EPCR. Furthermore, nicotine had no influence on TM and EPCR. In conclusion, the present study supports a novel molecular mechanism of cigarette smoking‑associated thrombosis by the decreased expression of TM. Further studies are required to identify specific components in CSE responsible for decreasing TM expression and its associated consequences.” (Wei Y et al. 2018).

“OBJECTIVE: Green Tobacco Sickness (GTS) is an occupational illness caused by dermal absorption of nicotine from tobacco leaves. It affects thousands of farm workers worldwide. Brazil is the second tobacco producer in the world; despite this, there are few studies on GTS among Brazilian harvesters. This study aimed to determine the prevalence of GTS among a population of tobacco workers from a producing area in northeastern Brazil and investigate whether the occurrence of the disease was influenced by factors such age, gender and smoking status. In addition, it was investigated if there was association between the onset of GTS and genetic polymorphisms in genes that encode some detoxification enzymes. A semi-structured questionnaire was used to collect demographic, behavioral and occupational data from the referred workers. Polymorphisms were tested through the Polymerase Chain Reaction technique. RESULTS: The total prevalence of GTS found was 56.9%, with a significant difference between genders (71.7% for women and 35.3% for men, p < 0.0001). No association was identified between the investigated polymorphisms and GTS. This study confirms the occurrence of GTS among tobacco harvesters in Brazil with high prevalence. The investigation suggests the need to take preventive measures to protect tobacco workers against this disease.” (da Mota et al. 2018).

“Acute exposure to nicotinic agonists induces myotoxicity in zebrafish embryos. The main goal of this work was to evaluate the potential myotoxicity of nicotine acetylcholine receptor agonists on adult zebrafish muscle tissue by using nicotine as a model compound. Liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) datasets were processed with different chemometric tools based on the selection of Regions of Interest (ROI) and Multivariate Curve-Resolution (ROI-MCR procedure) Alternating Least Squares (ALS) for the analysis of different exposure experiments. Analysis of Variance Simultaneous Component Analysis (ASCA) of changes on metabolite peak profile areas showed significant nicotine concentration and exposure time-dependent changes, clearly differentiating between exposed and non-exposed samples and between short (2 h) and long exposure times (6 h or 24 h). Most of the changes observed in the concentrations of different metabolites are probably secondary to the observed hyperlocomotion, as they have been also observed in humans after strenuous muscular exercise. The absence of myotoxicity might be related with the reduced calcium permeability of adult muscle-type nicotinic acetylcholine receptors (nAChRs).” (Gomez-Canela et al. 2018).

“BACKGROUND/AIM: We have previously reported that simvastatin exhibits antioxidant properties via extracellular signal-regulated kinase (ERK)/cAMP-response element binding (CREB) protein-dependent induction of heme oxygenase-1 (HO1) and chronic nicotine exposure inhibits ERK/CREB signaling in renal proximal tubule cells (through p66shc). Herein, whether nicotine dampens simvastatin-dependent HO1 induction was determined. MATERIALS AND METHODS: Renal proximal tubule (NRK52E) cells were pre-treated with 200 μM nicotine for 24 h followed by 10 μM simvastatin. Promoter activity of HO1 and manganese superoxide dismutase (MnSOD) and activation of CREB and ERK (via ELK1) were determined in luciferase reporter assays. CREB and p66shc were modulated via genetic means. RESULTS: Nicotine suppressed simvastatin-dependent activation of HO1 and MnSOD promoters and activity of CREB and ELK1 via p66shc. Overexpression of CREB or knockdown of p66shc restored simvastatin-dependent induction of HO1 and MnSOD in the presence of nicotine. CONCLUSION: Antioxidant efficiency of simvastatin might be significantly lessened in smokers/E-cigarette users.” (Arany et al. 2018).

“Maternal nicotine (NIC) exposure causes overweight, hyperleptinemia and metabolic disorders in adult offspring. Our study aims to explore the underlying mechanism of perinatal NIC exposure increases obesity susceptibility in adult female rat offspring. In our model, we found that adult NIC-exposed females presented higher body weight and subcutaneous and visceral fat mass, as well as larger adipocytes, while no change was found in food intake. Serum profile showed a higher serum glucose, insulin and leptin levels in NIC-exposed females. In adipose tissue and liver, the leptin signaling pathway was blocked at 26 weeks, presented lower Janus tyrosine kinase 2 and signal transducer and activator of transcription 3 gene expression, higher suppressor of cytokine signaling 3 gene expression (in adipose tissue) and lower leptin receptors gene expression (in liver), indicating that peripheral leptin resistance occurred in NIC-exposed adult females. In female rats, the expression of lipolysis genes was affected dominantly in adipose tissue, but lipogenesis genes was affected in liver. Furthermore, the glucose and insulin tolerance tests showed a delayed glucose clearance and a higher area under the curve in NIC-exposed females. Therefore, perinatal NIC exposure programed female rats for adipocyte hypertrophy and obesity in adult life, through the leptin resistance in peripheral tissue.” (Zhang et al. 2018).

# *6.* *Functional effects on*

## *6.1.* *Broncho/pulmonary system*

“Store‑operated calcium entry (SOCE) is critical for regulating the proliferation and metastasis of various cancer types. The present study aimed to investigate the role of SOCE on nicotine‑promoted proliferation of non‑small cell lung cancer (NSCLC) A549 cells. Cell proliferation was evaluated by BrdU incorporation assay. The SOCE and basal [Ca2+]i in NSCLC A549 cells were determined using Fura‑2 fluorescence microscopy. The mRNA and protein expression levels were determined by real‑time quantitative PCR and western blotting, respectively. The results demonstrated that, in A549 cells, the detectable store‑operated calcium channel (SOCC) components were TRPC proteins 1, 3, 4 and 6 and Orail, among which TRPC1, TRPC6 and Orai1 are expressed at relatively high levels with TRPC3 and TRPC4 at relatively low levels. Nicotine upregulated the mRNA and protein expression of TRPC1, TRPC6 and Orai1, increased basal [Ca2+]i and enhanced SOCE. Promotion of cell proliferation but not migration was observed in the nicotine‑treated cells, which was inhibited by SOCE inhibitor SKF‑96365. Furthermore, nicotine upregulated HIF‑1α expression in the A549 and NCI‑H292 cells. Silencing of HIF‑1α abrogated the increases in TRPCs and Orail and reversed the increases in basal [Ca2+]i and SOCE. Meanwhile, suppression of proliferation was observed in cells following HIF‑1α silencing. In conclusion, the results indicate that nicotine promotes lung cancer cell proliferation likely by upregulating HIF‑1α and SOCC components and therefore enhancing SOCE and increasing basal [Ca2+]i.” (Wang Y et al. 2018).

“Long-term exposure to cigarette smoke induces severe injuries to respiratory system through several mechanisms, some of them are well defined, but many others are not yet elucidated. Beside its classical role in nervous system, we have previously shown that Nerve Growth Factor (NGF) and its receptors have a crucial role in airway inflammatory diseases, such as Chronic Obstructive Pulmonary Disease. To expand our knowledge about the relevance of NGF and its receptors in airway diseases induced by cigarette smoking, we exposed for 16 weeks the bronchial epithelial cell line BEAS-2B to sub-toxic concentrations of whole cigarette smoke extract or pure nicotine. Viability, cell cycle gene expression, cell morphology and migration ability were tested and compared to NGF release and gene expression. Modulation of its receptors TrKA and p75NTR was also analyzed. The present study shows that long term exposure of BEAS-2B cells to cigarette smoke extract or nicotine induces: (A) differences: in cell viability, in the expression of cell cycle-related genes, in NGF release and in gene expression of NGF and its receptors; (B) similarities: in morphology and migration ability. Taken together, our data provide new insights about the biological role of NGF and its receptors in airway diseases induced by long-term cigarette smoking and, finally, our data evidence the opportunity not to use nicotine lozenges or e-cigarettes as anti smoking replacement therapy in patients with a previous airway disease according to the ability of nicotine to increase the amount of the pro-inflammatory cytokine NGF into the bronchial environment.” (Stabile et al. 2018).

“The use of novel tobacco- and nicotine-containing vapor products that do not combust tobacco leaves is on the rise worldwide. The emissions of these products typically contain lower numbers and levels of potentially harmful chemicals compared with conventional cigarette smoke. These vapor products may therefore elicit fewer adverse biological effects. We compared the effects of emissions from different types of such products, i.e., our proprietary novel tobacco vapor product (NTV), a commercially available heat-not-burn tobacco product (HnB), and e-cigarette (E-CIG), and a combustible cigarette in a human bronchial epithelial cell line. The aqueous extract (AqE) of the test product was prepared by bubbling the produced aerosol into medium. Cells were exposed to the AqEs of test products, and then glutathione oxidation, Nrf2 activation, and secretion of IL-8 and GM-CSF were examined. We found that all endpoints were similarly perturbed by exposure to each AqE, but the effective dose ranges were different between cigarette smoke and the tobacco- and nicotine-containing vapors. These results demonstrate that the employed assays detect differences between product exposures, and thus may be useful to understand the relative potential biological effects of tobacco- and nicotine-containing products.” (Munakata et al. 2018).

“The present study was designed to explore the role of transient receptor potential canonical 3 (TRPC3) in nicotine-induced chronic obstructive pulmonary disease (COPD) and its underlying mechanism. In this study, the expression and localization of α5 nicotinic acetylcholine receptor (α5-nAchR) in lung tissues were determined by western blotting and immunohistochemistry. The quantitative real-time PCR (qRT-PCR) analysis was performed to examine the mRNA expression levels of α5-nAchR and TRPC3 in human airway smooth muscle cells (HASMCs). Cell viability was assessed by CCK-8 assay. Proliferation was detected by cell counting and EdU immunofluorescent staining. Fluorescence calcium imaging was carried out to measure cytosolic Ca2+ ([Ca2+]cyt) concentration. The results showed that the α5-nAchR and TRPC3 expressions were significantly up-regulated in lung tissues of COPD smokers. Nicotine promoted HASMC proliferation, which was accompanied by elevated α5-nAchR and TRPC3 expressions, basal [Ca2+]cyt, store-operated calcium entry (SOCE) and the rate of Mn2+ quenching in HASMCs. Further investigation indicated that nicotine-induced Ca2+ response and TRPC3 up-regulation was reversibly blocked by small interfering RNA (siRNA) suppression of α5-nAChR. The knockdown of TRPC3 blunted Ca2+ response and HASMC proliferation induced by nicotine. In conclusion, nicotine-induced HASMC proliferation was mediated by TRPC3-dependent calcium entry via α5-nAchR, which provided a potential target for treatment of COPD.” (Jiang et al. 2019).

“INTRODUCTION: Nicotine stimulates fibroblast proliferation while increasing inflammation and fibrosis of tissues. The cannabinoid receptor 1 (CB1R) is mainly located in the CNS, while cannabinoid receptor 2 (CB2R) is located in the immune cells within the body. CB2R regulates inflammatory processes and fibroblast function. PURPOSE: We investigated the impact of CB2R agonist, JWH 133 and the antagonist, AM630 on lung tissue, applied directly before nicotine application. MATERIAL AND METHODS: 40 mice were placed into 4 groups. The experimental groups received nicotine intraperitoneally at a dose of 0.05 mg/kg of body weight (BW) for 14 days. Group B also received AM630 (0.5mg/kg of BW), while Group A was administered with JWH133 (1 mg/kg of BW). Group N received nicotine alone. The Control group C received 0.9% NaCl. After decapitation, lung tissues were stained with H&E, Trichrome Masson's method, and IHC against CTGF and α-SMA. The digital image processing system Image J with the IHC profiler plugins was then employed, optical density and IHC optical density score were calculated. RESULTS: In the N group, an increase in the thickness of alveolar spaces (9.16 SD4.95µm vs. 4.77SD2.99µm in the C group), leukocytes infiltration and collagen deposition has been observed (OD: 0.20 SD0.0vs 0.07SD0.04 in the C group). In the B group, the alveolar space thickness has been the highest (11.57SD8.13µm). Furthermore, in this group, hyperaemia, destruction of lung structure, hyperplasia of II type pneumocyte and interstitial fibrosis has been observed (OD: 0.23 SD0.08). In contrast, the lung tissue of the A group has had normal structure and the thinnest alveolar septum (3.88 SD2.64µm). The expression of CTGF and α-SMA has been the highest in the B group. CONCLUSION: Nicotine induces interstitial lung fibrosis that is enhanced by the CB2R antagonist and diminished by the CB2R agonist. Therefore, the CB2R agonist may offer a protection against fibrosis.” (Wawryk-Gawda et al. 2018).

“E-cigarette smoke delivers stimulant nicotine as aerosol without tobacco or the burning process. It contains neither carcinogenic incomplete combustion byproducts nor tobacco nitrosamines, the nicotine nitrosation products. E-cigarettes are promoted as safe and have gained significant popularity. In this study, instead of detecting nitrosamines, we directly measured DNA damage induced by nitrosamines in different organs of E-cigarette smoke-exposed mice. We found mutagenic O6-methyldeoxyguanosines and γ-hydroxy-1,N2 -propano-deoxyguanosines in the lung, bladder, and heart. DNA-repair activity and repair proteins XPC and OGG1/2 are significantly reduced in the lung. We found that nicotine and its metabolite, nicotine-derived nitrosamine ketone, can induce the same effects and enhance mutational susceptibility and tumorigenic transformation of cultured human bronchial epithelial and urothelial cells. These results indicate that nicotine nitrosation occurs in vivo in mice and that E-cigarette smoke is carcinogenic to the murine lung and bladder and harmful to the murine heart. It is therefore possible that E-cigarette smoke may contribute to lung and bladder cancer, as well as heart disease, in humans.” (Lee et al. 2018).

“Electronic (e)-cigarettes theoretically may be safer than conventional tobacco. However, our prior studies demonstrated direct adverse effects of e-cigarette vapor (EV) on airway cells, including decreased viability and function. We hypothesize that repetitive, chronic inhalation of EV will diminish airway barrier function, leading to inflammatory protein release into circulation, creating a systemic inflammatory state, ultimately leading to distant organ injury and dysfunction. C57BL/6 and CD-1 mice underwent nose only EV exposure daily for 3-6 mo, followed by cardiorenal physiological testing. Primary human bronchial epithelial cells were grown at an air-liquid interface and exposed to EV for 15 min daily for 3-5 days before functional testing. Daily inhalation of EV increased circulating proinflammatory and profibrotic proteins in both C57BL/6 and CD-1 mice: the greatest increases observed were in angiopoietin-1 (31-fold) and EGF (25-fold). Proinflammatory responses were recapitulated by daily EV exposures in vitro of human airway epithelium, with EV epithelium secreting higher IL-8 in response to infection (227 vs. 37 pg/ml, respectively; P < 0.05). Chronic EV inhalation in vivo reduced renal filtration by 20% ( P = 0.017). Fibrosis, assessed by Masson's trichrome and Picrosirius red staining, was increased in EV kidneys (1.86-fold, C57BL/6; 3.2-fold, CD-1; P < 0.05), heart (2.75-fold, C57BL/6 mice; P < 0.05), and liver (1.77-fold in CD-1; P < 0.0001). Gene expression changes demonstrated profibrotic pathway activation. EV inhalation altered cardiovascular function, with decreased heart rate ( P < 0.01), and elevated blood pressure ( P = 0.016). These data demonstrate that chronic inhalation of EV may lead to increased inflammation, organ damage, and cardiorenal and hepatic disease.” (Crotty et al. 2018).

“Electronic cigarette (e-cigarette) usage in the USA has drastically increased in the past 5 years due to age restrictions on conventional cigarettes, aggressive marketing and a perception that e-cigarettes are a healthy alternative. E-cigarettes contain nicotine, water, glycerol, propylene glycol and optional flavouring. On inhalation, the device heats the ingredients into a vapour. While tobacco cigarette smoke is known to cause deleterious effects on the cardiovascular system, angiogenesis and skin capillary perfusion by causing direct injury to blood vessel walls, increased platelet aggregation, microvascular thrombosis and inflammation, the consequences of e-cigarette vapour exposure on the lung are still largely unexplored. Recently, Lerner et al 2015. reported that vapours produced by e-cigarettes and e-cigarette fluids with flavourings induced toxicity, oxidative stress and inflammatory response in human bronchial airway epithelial cells (H292) and fetal lung fibroblasts (HFL1) as well as mouse lung. Garcia-Arcos et al. 2016 showed that the aerosolised nicotine-containing e-cigarette fluid increased airway hyperreactivity, distal airspace enlargement, mucin production, and cytokine and protease expression in mice, implying potential dangers of nicotine inhalation during e-cigarette use. The inflammatory response to e-cigarette use involved increased neutrophil activation and mucus production, and decreased mucociliary clearance. In human embryonic and mouse neural stem cells, human pulmonary fibroblasts, and skin and lung cells, cytotoxicity of e-cigarette vapour was correlated with the number and concentration of chemicals used to flavour the fluids. We recently showed in the skin flap survival model in vivo that nicotine-containing e-cigarette vapour is just as harmful to the microcirculation as tobacco cigarette smoke.” (Reinikovaite eta l. 2018).

“Recent advancements in in vitro exposure systems and cell culture technology enable direct exposure to cigarette smoke (CS) of human organotypic bronchial epithelial cultures. MucilAir organotypic bronchial epithelial cultures were exposed, using a Vitrocell exposure system, to mainstream aerosols from the 3R4F cigarette or from a recently developed novel tobacco vapor product (NTV). The exposure aerosol dose was controlled by dilution flow and the number of products smoked; there were five exposure conditions for 3R4F smoke and three for NTV vapor. The amount of nicotine delivered to the tissues under each condition was analyzed and that of the total particulate matter (TPM) was estimated using nicotine data. The nicotine dose was similar for the two products at the highest dose, but the estimated TPM levels from the NTV were 3.7 times the levels from the 3R4F. Following 3R4F smoke exposure, a dose dependent increase was observed in cytotoxicity, cytokine secretion, and differential gene expression. However, no changes were detected in these endpoints following NTV vapor exposure, suggesting the biological effects of NTV vapor are lower than those of conventional combustible CS. Our study design, which includes collection of biological data and dosimetry data, is applicable to assessing novel tobacco products.” (Ishikawa et al. 2018).

“Objective: To investigate the molecular mechanism of contractility dysfunction of human bronchial smooth muscle cells induced by nicotine. Methods: Primary human bronchial smooth muscle cells were cultured in vitro. The cells were divided into a control group and a nicotine group which was treated with 10(-5) mol/L nicotine for 48 h and transfected with or without α7nAChR-siRNA (The siNC group, siNC + nicotine group and siα7nAChR + nicotine group). The effects of nicotine on the cell contractile function were examined by collagen gel shrinkage assay. The expressions of α7nAChR and TRPC6 protein in nicotine-treated human bronchial smooth muscle cells were detected by Western blotting. The change of intracellular calcium concentration by nicotine was detected by calcium ion imaging system.Data were analyzed by t test or single factor analysis of variance. Results: The area of collagen gel in the nicotine group (24±8)% was significantly lower than that in the control group (59±14)% (t=3.78, P<0.05). Compared with the control group, the expression of α7nAChR protein in nicotine-induced group (173±16)% was significantly higher than that of controls 100±0)%, t=-6.848, P<0.05. Compared with the siNC group [(72±10)%, (0.79±0.07), (0.41±0.04) and (0.17±0.02) respectively], the collagen gel area of siNC + nicotine group was significantly reduced by (37±10)%. However, the basal calcium level (1.04±0.02), store operated calcium entry level (SOCE, 0.68±0.03) and receptor operated calcium entry level (ROCE, 0.36±0.02) were remarkably elevated in the nicotine treated group (all P<0.05). Furthermore, compared with siNC + nicotine group, the area of collagen gel in siα7nAChR + nicotine group was significantly increased (62±10)%, and the basal calcium level (0.78±0.06), SOCE level (0.39±0.05) and ROCE level (0.15±0.02) were significantly reduced (all P<0.05). Conclusions: Nicotine can increase the expression of TRPC6 protein, SOCE and ROCE level, and increase the intracellular calcium concentration by upregulating the expression of α7nAChR protein, thereby promoting smooth muscle cell contraction.” (Hong et al. 2018).

“The electronic cigarette (e-cig) has been suggested as a safer alternative to tobacco cigarettes. However, the health effects of e-cigs on the airways have not been fully investigated. Nicotine, the primary chemical constituent of the e-cig aerosol, has been shown to stimulate vagal bronchopulmonary C-fiber sensory nerves, which upon activation can elicit vigorous pulmonary defense reflexes, including airway constriction. In this study, we investigated the bronchomotor response to e-cig inhalation challenge in anesthetized guinea pigs and the mechanisms involved in regulating these responses. Our results showed that delivery of a single puff of e-cig aerosol into the lung triggered immediately a transient bronchoconstriction that sustained for >2 min. The increase in airway resistance was almost completely abolished by a pretreatment with either intravenous injection of atropine or inhalation of aerosolized lidocaine, suggesting that the bronchoconstriction was elicited by cholinergic reflex mechanism and stimulation of airway sensory nerves was probably involved. Indeed, electrophysiological recording further confirmed that inhalation of e-cig aerosol exerted a pronounced stimulatory effect on vagal bronchopulmonary C-fibers. These effects on airway resistance and bronchopulmonary C-fiber activity were absent when the e-cig aerosol containing zero nicotine was inhaled, indicating a critical role of nicotine. Furthermore, a pretreatment with nicotinic acetylcholine receptor antagonists by inhalation completely prevented the airway constriction evoked by e-cig aerosol inhalation. In conclusion, inhalation of a single puff of e-cig aerosol caused a transient bronchoconstriction that was mediated through cholinergic reflex and triggered by a stimulatory effect of nicotine on vagal bronchopulmonary C-fiber afferents.” (Khosravi et al. 2018).

“The long-term impact of nicotine from e-cigarettes on lung tissue is not yet known and may be different from its impact systemically.

A recent animal study suggested that nicotine can have adverse effects on the lung (95). The study used very large doses of nicotine administered intermittently for four months. The organ damage could have been due to systemic poisoning and may not be relevant to exposure in smokers and EC users (96). Nevertheless, the effects of inhaled nicotine on lung function in humans require further investigation.” (Public Health England, 2018)

“Nicotine is a major component of cigarette smoke. It causes addiction and is used clinically to aid smoke cessation. The aim of the present study is to investigate the effect of nicotine on lipopolysaccharide (LPS)-induced airway hyperreactivity (AHR) and to explore the potential involvement of neuronal mechanisms behind nicotine's effects in murine models in vivo and in vitro. BALB/c mice were exposed to nicotine in vivo via subcutaneous Alzet osmotic minipumps containing nicotine tartate salt solution (24 mg·kg-1·day-1) for 28 days. LPS (0.1 mg/ml, 20 µl) was administered intranasally for 3 consecutive days during the end of this period. Lung functions were measured with flexiVent. For the in vitro experiments, mice tracheae were organcultured with either nicotine (10 μM) or vehicle (DMSO, 0.1%) for 4 days. Contractile responses of the tracheal segments were measured in myographs following electric field stimulation (EFS; increasing frequencies of 0.2 to 12.8 Hz) before and after incubation with 10 µg/ml LPS for 1 h. Results showed that LPS induced AHR to methacholine in vivo and increased contractile responses to EFS in vitro. Interestingly, long-term nicotine exposure markedly dampened this LPS-induced AHR both in vitro and in vivo. Tetrodotoxin (TTX) inhibited LPS-induced AHR but did not further inhibit nicotine-suppressed AHR in vivo. In conclusion, long-term nicotine exposure dampened LPS-induced AHR. The effect of nicotine was mimicked by TTX, suggesting the involvement of neuronal mechanisms. This information might be used for evaluating the long-term effects of nicotine and further exploring of how tobacco products interact with bacterial airway infections.” (Xu and Cardell 2017).

“BACKGROUND: Tobacco-related chronic lung diseases are characterized by alterations in lung architecture leading to decreased lung function. Knowledge of the exact mechanisms involved in tobacco-induced tissue remodeling and inflammation remains incomplete. We hypothesize that nicotine stimulates the expression of extracellular matrix proteins, leading to relative changes in lung matrix composition, which may affect immune cells entering the lung after injury. METHODS: Pulmonary fibroblasts from wildtype and α7 nicotinic acetylcholine receptor knockout (α7KO) mice were exposed to nicotine and examined for collagen type 1 mRNA and protein expression. Testing the potential role on immune cell function, pulmonary fibroblasts were retained in culture for 120 h. The fibroblasts were eliminated by osmotic lysis and the remaining matrix-coated dishes were washed thoroughly. U937 cells were incubated on the matrix-coated dishes for 24 h followed by evaluation of IL-1β gene expression. Wildtype or α7KO C57BL/6 mice (female, 8-12 weeks) were fed normal diet and exposed to nicotine in their drinking water (100 μg/ml) for 8-12weeks. Lungs were processed for mRNA, protein, and histology. Statistical significance was determined at p ≤ .05 by two-tailed test or 2-way ANOVA with Bonferroni posttest. RESULTS: We found that nicotine stimulated collagen type I mRNA and protein expression in a dose-dependent manner and up to 72 h in primary lung fibroblasts. The stimulatory effect of nicotine was inhibited in α7KO primary lung fibroblasts. Testing the potential role of these events on immune cell function, U937 monocytic cells were cultured atop matrices derived from nicotine-treated lung fibroblasts. These cells expressed more IL-1β than those cultured atop matrices derived from untreated fibroblasts, and antibodies against the α2β1 collagen integrin receptor inhibited the effect. Nicotine also stimulated fibroblast proliferation via MEK-1/ERK, unveiling a potentially amplifying pathway. In vivo, nicotine increased collagen type I expression was detected in wildtype, but not in α7KO mice. Wildtype mice showed increased collagen staining in lung, primarily around the airways. CONCLUSIONS: These observations suggest that nicotine stimulates fibroblast proliferation and their expression of collagen type I through α7 nAChRs, thereby altering the relative composition of the lung matrix without impacting the overall lung architecture; this may influence inflammatory responses after injury.” (Vicary et al. 2017).

“PURPOSE OF REVIEW: Vaping is gaining popularity in the USA, particularly among teens and young adults. While e-cigs are commonly represented as safer alternatives to tobacco cigarettes, little is known regarding the health effects of their short- or long-term use, especially in individuals with pre-existing respiratory diseases such as asthma. Flavored e-cig liquids (e-liquids) and e-cig aerosols contain airway irritants and toxicants that have been implicated in the pathogenesis and worsening of lung diseases. In this review, we will summarize existing data on potential health effects of components present in e-cig aerosols, such as propylene glycol, vegetable glycerin, nicotine, and flavorings, and discuss their relevance in the context of asthma. RECENT FINDINGS: Recent survey data indicate that adolescents with asthma had a higher prevalence of current e-cig use (12.4%) compared to their non-asthmatics peers (10.2%) and conveyed positive beliefs about tobacco products, especially e-cigs. Similarly, a study conducted among high school students from Ontario, Canada, indicated a greater likelihood of e-cig use in asthmatics as compared to their non-asthmatic peers. Availability of different flavorings is often cited as the main reason among youth/adolescents for trying e-cigs or switching from cigarettes to e-cigs. Occupational inhalation of some common food-safe flavoring agents is reported to cause occupational asthma and worsen asthmatic symptoms. Moreover, workplace inhalation exposures to the flavoring agent diacetyl have caused irreversible obstructive airway disease in healthy workers. Additionally, recent studies report that thermal decomposition of propylene glycol (PG) and vegetable glycerin (VG), the base constituents of e-liquids, produces reactive carbonyls, including acrolein, formaldehyde, and acetaldehyde, which have known respiratory toxicities. Furthermore, recent nicotine studies in rodents reveal that prenatal nicotine exposures lead to epigenetic reprogramming in the offspring, abnormal lung development, and multigenerational transmission of asthmatic-like symptoms. Comparisons of the toxicity and health effects of e-cigs and conventional cigarettes often focus on toxicants known to be present in cigarette smoke (CS) (i.e., formaldehyde, nitrosamines, etc.), as well as smoking-associated clinical endpoints, such as cancer, bronchitis, and chronic obstructive pulmonary disease (COPD). However, this approach disregards potential toxicity of components unique to flavored e-cigs, such as PG, VG, and the many different flavoring chemicals, which likely induce respiratory effects not usually observed in cigarette smokers.” (Clapp and Jaspers 2017).

## *6.2.* *Cardiovascular system*

“METHODS: Pregnant C57 mice drank nicotine- or cotinine-laced water for 6 wks from conception (NPRE = 2% saccharin + 100 μg nicotine/mL; CPRE = 2% saccharin + 10 μg cotinine/mL) or 3 wks after birth (CPOST = 2% saccharin + 30 μg cotinine/mL). Controls drank 2% saccharin (CTRL). At 17 ± 1 weeks (male pups; CTRL n = 6; CPOST n = 6; CPRE n = 8; NPRE n = 9), we assessed (i) cardiovascular control during sleep; (ii) arterial reactivity ex vivo; and (iii) expression of genes involved in arterial constriction/dilation. RESULTS: Blood cotinine levels recapitulated those of passive smoker mothers' infants. Pups exposed to cotinine exhibited (i) mild bradycardia - hypotension at rest (p < 0.001); (ii) attenuated (CPRE , p < 0.0001) or reverse (CPOST ; p < 0.0001) BP stress reactivity; (iii) adrenergic hypocontractility (p < 0.0003), low protein kinase C (p < 0.001) and elevated adrenergic receptor mRNA (p < 0.05; all drug-treated arteries); and (iv) endothelial dysfunction (NPRE only). CONCLUSION: Cotinine has subtle, enduring developmental consequences. Some cardiovascular effects of nicotine can plausibly arise via conversion into cotinine. Low-level exposure to this metabolite may pose unrecognised perinatal risks. Adults must avoid inadvertently exposing a foetus or infant to cotinine as well as nicotine.” (Bastianini et al. 2018).

“Cigarette smoking is a major risk factor for atherosclerosis and other cardiovascular diseases. Increasing evidence has demonstrated that nicotine impairs the cardiovascular system by targeting vascular endothelial cells, but the underlying mechanisms remain obscure. It is known that cell death and inflammation are crucial processes leading to atherosclerosis. We proposed that pyroptosis may be implicated in nicotine-induced atherosclerosis and therefore conducted the present study. We found that nicotine resulted in larger atherosclerotic plaques and secretion of inflammatory cytokines in ApoE-/- mice fed with a high-fat diet (HFD). Treatment of human aortic endothelial cells (HAECs) with nicotine resulted in NLRP3-ASC inflammasome activation and pyroptosis, as evidenced by cleavage of caspase-1, production of downstream interleukin (IL)-1β and IL-18, and elevation of LDH activity and increase of propidium iodide (PI) positive cells, which were all inhibited by caspase-1 inhibitor. Moreover, silencing NLRP3 or ASC by small interfering RNA efficiently suppressed nicotine-induced caspase-1 cleavage, IL-18 and IL-1β production, and pyroptosis in HAECs. Further experiments revealed that the nicotine-NLRP3-ASC-pyroptosis pathway was activated by reactive oxygen species (ROS), since ROS scavenger (N-acetyl-cysteine, NAC) prevented endothelial cell pyroptosis. We conclude that pyroptosis is likely a cellular mechanism for the pro-atherosclerotic property of nicotine and stimulation of ROS to activate NLRP3 inflammasome is a signaling mechanism for nicotine-induced pyroptosis.” (Wu et al. 2018).

“The present study investigated the influence of cigarette smoke extract (CSE) and nicotine on the expression of thrombomodulin (TM) and endothelial protein C receptor (EPCR) in human umbilical vein endothelial cells (HUVECs). Smoking is associated with intravascular thrombosis. As a vital anticoagulation cofactor, TM is located on the endothelial cell surface and regulates intravascular coagulation by binding to thrombin, hence activating protein C. Activated protein C is a natural anticoagulant that interacts with EPCR to enhance the function of anticoagulation system. The effects of CSE (0.5‑5%) and nicotine (10‑3‑10‑9 mol/l) on the expression of TM and EPCR in HUVECs were observed. Reverse transcription‑quantitative polymerase chain reaction and flow cytometric analysis techniques were used for detecting TM and EPCR mRNA and protein expression levels, respectively. After 6‑h exposure, TM protein and mRNA expression levels decreased in a dose‑dependent manner. Stimulation with 5% CSE for 0, 6, 10, 12 and 24 h led to a decrease in the levels of TM mRNA and protein over time, which reached a peak at 12 h. The levels were significantly reduced compared with the control group (P<0.001). However, CSE had no effect on EPCR. Furthermore, nicotine had no influence on TM and EPCR. In conclusion, the present study supports a novel molecular mechanism of cigarette smoking‑associated thrombosis by the decreased expression of TM. Further studies are required to identify specific components in CSE responsible for decreasing TM expression and its associated consequences.” (Wei Y et al. 2018).

“Estrogen-progestin oral contraceptives (COC) or tobacco smoking has been associated with hypertension and endothelial dysfunction resulting in increased risk of cardiovascular diseases (CVD). Contrasting effects of nicotine exposure on endothelial function have been reported. The effect of non-smoking nicotine exposure on endothelial dysfunction during COC treatment remains to be fully elucidated. We therefore, sought to determine the effects of nicotine exposure during COC treatment on endothelial dysfunction mediators and circulating corticosteroids. Female Wistar rats aged 10 weeks were given (po) vehicle, nicotine (1.0 mg/kg) with or without COC steroids (1.0 µg ethinylestradiol and 5.0 µg levonorgestrel) daily for 6 weeks. Nicotine exposure caused 113.3% increase in insulinemia whereas COC treatment led to 76.9% increased insulinemia compared with control. Furthermore, COC treatment or nicotine exposure led to glucose deregulation, insulin resistance, reduced nitric oxide bioavailability, elevated plasminogen activator inhibitor-1, uric acid, oxidative stress, atherogenic dyslipidemia, and corticosteroids. However, COC + NIC treatment led to 41.2% decrease in insulemina compared with COC-treated rats. Furthermore, all other alterations were alleviated by nicotine exposure in COC-treated female rats with the exception of corticosteroids.” (Michael and Olatunji 2018).

“The introduction of electronic cigarettes has led to widespread discussion on the cardiovascular risks compared to conventional smoking. We therefore conducted a randomized cross-over study of the acute use of three tobacco products, including a control group using a nicotine-free liquid. Fifteen active smokers were studied during and after smoking either a cigarette or an electronic cigarette with or without nicotine (eGo-T CE4 vaporizer). Subjects were blinded to the nicotine content of the electronic cigarette and were followed up for 2 hours after smoking a cigarette or vaping an electronic cigarette. Peripheral and central blood pressures as well as parameters of arterial stiffness were measured by a Mobil-O-Graph® device. The peripheral systolic blood pressure rose significantly for approximately 45 minutes after vaping nicotine-containing liquid (p<0.05) and for approximately 15 minutes after smoking a conventional cigarette (p<0.01), whereas nicotine-free liquids did not lead to significant changes during the first hour of follow-up. Likewise, heart rate remained elevated approximately 45 minutes after vaping an electronic cigarette with nicotine-containing liquid and over the first 30 minutes after smoking a cigarette in contrast to controls. Elevation of pulse wave velocity was independent from mean arterial pressure as well as heart rate in the electronic cigarette and cigarette groups. In this first of its kind trial, we observed changes in peripheral and central blood pressure and also in pulse wave velocity after smoking a cigarette as well as after vaping a nicotine-containing electronic cigarette. These findings may be associated with an increased long-term cardiovascular risk.” (Franzen et al. 2018).

“A reference made at a conference to a research letter (97) reported that nicotine in EC causes transient stiffening of arteries and the author claimed in a media release that this shows risks of vaping and that he would ‘not encourage the use of the devices’. This generated several headlines, such as the front page headline ‘Vaping as bad as fags: E-cigs seriously damage heart’ (McDermott, the Sun 2016, available at: https://www.thesun.co.uk/living/1693653/e-cigs-are-just-as-bad-for-your-heart-assmoking-fags-as-they-damage-key-blood-vessels-say-experts/) and other similar headlines ‘E-cigarettes are as bad for the heart as tobacco: Nicotine vapour damages blood vessels and raises risk of disease’; and ‘Vaping as bad for your heart as smoking cigarettes’ (http://www.telegraph.co.uk/news/2016/08/29/vaping-as-bad-for-your-heartas-smoking-cigarettes-study-finds/). It seems likely that this effect is due to the acute sympathetic activation induced by nicotine through the release of norepinephrine. However, the same author (98) had previously found that the same effect, but stronger and longer lasting, follows drinking coffee, and also after chronic consumption (99). Similar effects have also been observed among students who are sitting an exam or engage in other common activities that can result in mental stress (100).

A study using the UK Clinical Practice Research Datalink detected a shorter survival time in patients with pre-existing CVD who were prescribed NRT compared to those receiving stop-smoking advice only (16). The raw data, however, did not control for potential selection biases: for example, General Practitioners (GPs) may have a greater propensity to prescribe NRT to heavier smokers about whom they are concerned; similarly, smokers with more severe symptoms may be more willing to accept the prescription. The study also did not control for or assess the duration of NRT use – any past use was sufficient to categorise the patient as an NRT user. Further studies controlling for relevant covariates would be useful to clarify the above issues.

The RCP report indicated that short-term nicotine use does not result in ‘clinically significant harm’ and concluded that there was no evidence of any increase in the risk of heart attack, stroke or death from use of NRT in quit attempts.” (Public Health England, 2018)

“BACKGROUND: Reports showed that estrogen-progestin oral contraceptive (COC) or tobacco smoking causes increased risk of cardiovascular diseases (CVD) in premenopausal women. Studies also suggest that nicotine, a major tobacco alkaloid, may worsen or improve atherothrombotic CVD. Altered hemorheology, prothrombotic and pro-inflammatory biomarkers, have been implicated in the development of atherothrombotic CVD events. However, the effect of non-smoking nicotine exposure on these biomarkers during COC treatment is not yet established. We therefore sought to determine the effects of nicotine exposure during COC treatment on these biomarkers, and also tested the hypothesis that the nicotine effects would be glucocorticoid-dependent. METHODS: Female Sprague-Dawley rats aged 10 weeks were given (po) vehicle, low-dose nicotine (0.1mg/kg) or high-dose nicotine (1.0mg/kg) with or without COC steroids (5.0μg/kg ethinylestradiol and 25.0μg/kg levonorgestrel) daily for 6 weeks. RESULTS: COC treatment or nicotine exposure led to increased insulin resistance (IR), hemorheological (blood viscosity, hematocrit and plasma viscosity), prothrombotic (plasminogen activator inhibitor-1), pro-inflammatory (uric acid, C-reactive protein, neutrophil/lymphocyte and platelet/lymphocyte ratios) biomarkers and corticosterone. However, these effects except that on corticosterone were abrogated by nicotine exposure during COC treatment. CONCLUSIONS: Our study indicates that nicotine- or COC-induced IR may be mediated via inflammatory/thrombotic pathway. The results imply that nicotine exposure could impact negatively on atherothrombotic biomarkers in COC non-users, whereas the impact in COC users could be positive. The results also suggest that the anti-inflammatory, antithrombotic and blood viscosity-lowering effects of nicotine exposure during COC use is circulating glucocorticoid-independent.” (Olatunji et al. 2017).

“BACKGROUND: Since the introduction of the electronic e-cigarette a few years ago, its use has greatly increased. The liquid formulations used in these e-cigarettes contain nicotine in high concentrations; ingestion of these liquids can be fatal. CASE DESCRIPTION: A 42-year-old male was admitted to the Intensive Care ward due to cardiac arrest. The patient had ingested highly concentrated liquid nicotine, originating from a vial with liquid for e-cigarettes. When the ambulance personnel found the patient he did not have a pulse; following CPR and administration of adrenaline his pulse returned. Upon admission, the plasma nicotine level was high at 3.0 mg/l (reference values for a smoker are 0.01-0.05 mg/l) and the patient's neurological function was poor. The patient was treated symptomatically, but eventually died of a postanoxic encephalopathy. CONCLUSION: Nicotine e-liquids are highly concentrated. Intentional ingestion can lead to toxic levels of nicotine which are associated with cardiac arrhythmias or arrest. Because even a few millilitres can be lethal, nicotine intoxication due to e-liquid ingestion should be considered potentially life-threatening.” (van der Meer et al. 2017).

“Objectives: The aim was to investigate the effects of nicotine on neutrophil extracellular traps (NETs) formation in current and non-smokers and on a murine model of RA. Methods: We compared spontaneous and phorbol 12-myristate 13-acetate-induced NETosis between current and non-smokers by DNA release binding. Nicotine-induced NETosis from non-smokers was assessed by DNA release binding, NET-specific (myeloperoxidase (MPO)-DNA complex) ELISA and real-time fluorescence microscopy. We also used immunofluorescent staining to detect nicotinic acetylcholine receptors (nAChRs) on neutrophils and performed a functional analysis to assess the role of nAChRs in nicotine-induced NETosis. Finally, we investigated the effects of systemic nicotine exposure on arthritis severity and NETosis in the CIA mouse model. Results: Neutrophils derived from current smokers displayed elevated levels of spontaneous and phorbol 12-myristate 13-acetate-induced NETosis. Nicotine induced dose-dependent NETosis in ex vivo neutrophils from healthy non-smokers, and co-incubation with ACPA-immune complexes or TNF-α facilitated a synergistic effect on NETosis. Real-time fluorescence microscopy revealed robust formation of NET-like structures in nicotine-exposed neutrophils. Immunofluorescent staining demonstrated the presence of the α7 subunit of the nAChR on neutrophils. Stimulation of neutrophils with an α7-specific nAChR agonist induced NETosis, whereas pretreatment with an nAChR antagonist attenuated nicotine-induced NETosis. Nicotine administration to mice with CIA exacerbated inflammatory arthritis, with higher plasma levels of NET-associated MPO-DNA complex. Conclusion: We demonstrate that nicotine is a potent inducer of NETosis, which may play an important role in accelerating arthritis in the CIA model. This study generates awareness of and the mechanisms by which nicotine-containing products, including e-cigarettes, may have deleterious effects on patients with RA.” (Lee et al. 2017).

## *6.3.* *Nervous system*

“Smoking is a preventable risk factor for stroke and smoking-derived nicotine exacerbates post-ischemic damage via inhibition of estrogen receptor beta (ER-&beta;) signaling in the brain of female rats. ER-&beta; regulates inflammasome activation in the brain. Therefore, we hypothesized that chronic nicotine exposure activates the inflammasome in the brain, thus exacerbating ischemic brain damage in female rats. To test this hypothesis, adult female Sprague-Dawley rats (6⁻7 months old) were exposed to nicotine (4.5 mg/kg/day) or saline for 16 days. Subsequently, brain tissue was collected for immunoblot analysis. In addition, another set of rats underwent transient middle cerebral artery occlusion (tMCAO; 90 min) with or without nicotine exposure. One month after tMCAO, histopathological analysis revealed a significant increase in infarct volume in the nicotine-treated group (64.24 &plusmn; 7.3 mm³; mean &plusmn; SEM; n = 6) compared to the saline-treated group (37.12 &plusmn; 7.37 mm³; n = 7, p < 0.05). Immunoblot analysis indicated that nicotine increased cortical protein levels of caspase-1, apoptosis-associated speck-like protein containing a CARD (ASC) and pro-inflammatory cytokines interleukin (IL)-1&beta; by 88% (p < 0.05), 48% (p < 0.05) and 149% (p < 0.05), respectively, when compared to the saline-treated group. Next, using an in vitro model of ischemia in organotypic slice cultures, we tested the hypothesis that inhibition of nicotine-induced inflammasome activation improves post-ischemic neuronal survival. Accordingly, slices were exposed to nicotine (100 ng/mL; 14⁻16 days) or saline, followed by treatment with the inflammasome inhibitor isoliquiritigenin (ILG; 24 h) prior to oxygen-glucose deprivation (OGD; 45 min). Quantification of neuronal death demonstrated that inflammasome inhibition significantly decreased nicotine-induced ischemic neuronal death. Overall, this study shows that chronic nicotine exposure exacerbates ischemic brain damage via activation of the inflammasome in the brain of female rats.” (d’Adesky et al. 2018).

“Parkinson's disease (PD) is characterized by the degeneration of the dopaminergic neurons in the substantia nigra pars compacta (SNpc). Clinical and experimental evidence suggest that the activation of the nicotinic acetylcholine receptor (nAChR) could be protective for PD. In this study, we investigated the neuroprotective capacity of nicotine in a rat PD model. Considering that iron metabolism has been implicated in PD pathophysiology and nicotine has been described to chelate this metal, we also studied the effect of nicotine on the cellular labile iron pool (LIP) levels. Rotenone (1 μg) was unilaterally injected into the median forebrain bundle to induce the degeneration of the nigrostriatal pathway. Nicotine administration (1 mg/K, s.c. daily injection, starting 5 days before rotenone and continuing for 30 days) attenuated the dopaminergic cell loss in the SNpc and the degeneration of the dopaminergic terminals provoked by rotenone, as assessed by immunohistochemistry. Furthermore, nicotine partially prevented the reduction on dopamine levels in the striatum and improved the motor deficits, as determined by HPLC-ED and the forelimb use asymmetry test, respectively. Studies in primary mesencephalic cultures showed that pretreatment with nicotine (50 μM) improved the survival of tyrosine hydroxylase-positive neurons after rotenone (20 nM) exposure. Besides, nicotine induced a reduction in the LIP levels assessed by the calcein dequenching method only at the neuroprotective dose. These effects were prevented by addition of the nAChRs antagonist mecamylamine (100 μM). Overall, we demonstrate a neuroprotective effect of nicotine in a model of PD in rats and that a reduction in iron availability could be an underlying mechanism.” (Mouhape et al. 2019).

“Introduction: The main purpose of this study was to investigate the effects and possible mechanisms of nicotine pre-treatment on postoperative cognitive dysfunction (POCD) in aged rats. Methods: Nicotine (0.5 mg/kg) was given i.p. immediately after anesthesia induction. After the Morris water maze test was used to evaluate the rats' spatial learning and memory, serum and hippocampal tissues were harvested 1 and 3 days after intervention. Inflammatory cytokines in the serum were evaluated by Enzyme-linked Immunosorbent Assay (ELISA). Brain-derived neurotrophic factor (BDNF), p-TrkB, neuroinflammation cytokines, NF-κB p65, and cleaved caspase-3 were measured by western blotting; neuronal apoptosis in the hippocampal CA1 region was also evaluated by TUNEL staining. Results: We found that nicotine markedly attenuated the POCD and reduced the elevated levels of inflammatory cytokines in the serum, including IL-1β and high mobility group box-1 (HMGB1), on postoperative day 1. Additionally, nicotine suppressed the surgery-induced release of IL-1β, TNF-ɑ, HMGB1, and NF-κB p65 in the hippocampus on postoperative day 1 and day 3. In addition, operated rats displayed lower BDNF and p-TrkB in the hippocampus on postoperative day 1, returning to baseline by postoperative day 3. However, nicotine pre-treatment clearly reversed the surgical stress-induced decrease in both BDNF and p-TrkB expression in the hippocampus. Furthermore, nicotine pre-treatment significantly alleviated the surgery-induced increase in the neuronal apoptosis in the hippocampus on postoperative day 1 and day 3. Conclusions: Our results showed that nicotine-induced neuroprotection against POCD may involve activation of the BDNF/TrkB signaling pathway and inhibition of the NF-κB signaling pathway. Implications: Nicotine has long been considered a potent therapeutic agent for neuroprotection. This study reported the positive effect of nicotine treatment on cognitive dysfunction after partial hepatectomy in aged rats. Furthermore, the underlying mechanism may involve activation of the BDNF/TrkB signaling pathway and inhibition of the NF-κB signaling pathway in the hippocampus.” (Wei P et al. 2018).

“The adverse effects of prenatal nicotine and alcohol exposure on human reproductive outcomes are a major scientific and public health concern. In the United States, substantial percentage of women (20-25%) of childbearing age currently smoke cigarettes and consume alcohol, and only a small percentage of these individuals quit after learning of their pregnancy. However, there are very few scientific reports on the effect of nicotine in prenatal alcohol exposure on the cerebellum of the offspring. Therefore, this study was conducted to investigate the cerebellar neurotoxic effects of nicotine in a rodent model of Fetal Alcohol Spectrum Disorder (FASD). In this study, we evaluated the behavioral changes, biochemical markers of oxidative stress and apoptosis, mitochondrial functions and the molecular mechanisms associated with nicotine in prenatal alcohol exposure on the cerebellum. Prenatal nicotine and alcohol exposure induced oxidative stress, did not affect the mitochondrial functions, increased the monoamine oxidase activity, increased caspase expression and decreased ILK, PSD-95 and GLUR1 expression without affecting the GSK-3β. Thus, our current study of prenatal alcohol and nicotine exposure on cerebellar neurotoxicity may lead to new scientific perceptions and novel and suitable therapeutic actions in the future.” (Bhattacharya et al 2018).

“Cigarette smoke has significant toxic effects on the immune system, and increases the risk of developing autoimmune diseases; one immunosuppressive effect of cigarette smoke is that it inhibits the T cell-stimulating, immunogenic properties of myeloid dendritic cells (DCs). As the functions of DCs are regulated by intra-cellular signaling pathways, we investigated the effects of cigarette smoke extract (CSE) and nicotine on multiple signaling molecules and other regulatory proteins in human DCs to elucidate the molecular basis of the inhibition of DC maturation and function by CSE and nicotine. Maturation of monocyte-derived DCs was induced with the TLR3-agonist poly I:C or with the TLR4-agonist lipopolysaccharide, in the absence or presence of CSE or nicotine. Reverse-phase protein microarray was used to quantify multiple signaling molecules and other proteins in cell lysates. Particularly in poly I:C-matured DCs, cigarette smoke constituents and nicotine suppressed the expression of signaling molecules associated with DC maturation and T cell stimulation, cell survival and cell migration. In conclusion, constituents of tobacco smoke suppress the immunogenic potential of DCs at the signaling pathway level.” (Alkhattabi et al. 2018).

“Prenatal nicotine exposure (PNE) can cause hypersensitivity of hypothalamic-pituitary-adrenal (HPA) axis in offspring with intrauterine growth retardation. The purpose of this study was to explore the original mechanism of intrauterine development that mediates hypersensitivity of the HPA axis in offspring due to PNE. Pregnant Wistar rats were injected subcutaneously with 2 mg/kg·d of nicotine on the 9th to the 20th gestational day (GD9-GD20) and the fetuses were extracted at GD20. Compared with the control group, fetal rats by PNE showed increased hippocampal apoptosis, reduced synaptic plasticity and downregulation of the brain-derived neurotrophic factor (BDNF) pathway, whereas glutamic acid decarboxylase 67 (GAD67) expression was upregulated. Rat fetal hippocampal H19-7/IGF1R cell lines were treated with different concentrations of nicotine (1, 10 and 100 μM) for 3 days, the extracellular fluid glutamate (Glu) level increased and similar effects were observed as in vivo. Intervention treatments caused the opposite results. These results indicated that PNE downregulates the BDNF pathway and mediates the hippocampal excitotoxicity; then, the compensatory upregulation of GAD67 causes the imbalance of signal output in the fetal hippocampus. The negative feedback regulation of the paraventricular hypothalamic nucleus by the hippocampus is unbalanced, eventually causing hypersensitivity of the HPA axis of the offspring.” (Pei et al. 2019).

“Nicotine has been recognized to trigger various neuronal disabilities in the fetal brain and long-lasting behavioral deficits in offspring. However, further understanding of fetal brain development under nicotine exposure is challenging due to the limitations of existing animal models. Here, we create a new brain organoid-on-a-chip system derived from human induced pluripotent stem cells (hiPSCs) that allows us to model neurodevelopmental disorders under prenatal nicotine exposure (PNE) at early stages. The brain organoid-on-a-chip system facilitates 3D culture, in situ neural differentiation, and self-organization of brain organoids under continuous perfused cultures in a controlled manner. The generated brain organoids displayed well-defined neural differentiation, regionalization, and cortical organization, which recapitulates the key features of the early stages of human brain development. The brain organoids exposed to nicotine exhibited premature neuronal differentiation with enhanced expression of the neuron marker TUJ1. Brain regionalization and cortical development were disrupted in the nicotine-treated organoids identified by the expressions of forebrain (PAX6 and FOXG1), hindbrain (PAX2 and KROX20) and cortical neural layer (preplate TBR1 and deep-layer CTIP2) markers. Moreover, the neurite outgrowth showed abnormal neuronal differentiation and migration in nicotine-treated brain organoids. These results suggest that nicotine exposure elicits impaired neurogenesis in early fetal brain development during gestation. The established brain organoid-on-a-chip system provides a promising platform to model neurodevelopmental disorders under environmental exposure, which can be extended for applications in brain disease studies and drug testing.” (Wang Y et al. 2018b).

“The current study analyzed the effects of environmental enrichment versus isolation housing on the behavioral sensitization to nicotine in the neonatal quinpirole (NQ; dopamine D2-like agonist) model of dopamine D2 receptor supersensitivity, a rodent model of schizophrenia. NQ treatment in rats increases dopamine D2 receptor sensitivity throughout the animal's lifetime, consistent with schizophrenia. Animals were administered NQ (1 mg/kg) or saline (NS) from postnatal day (P)1 to P21, weaned, and immediately placed into enriched housing or isolated in wire cages throughout the experiment. Rats were behaviorally sensitized to nicotine (0.5 mg/kg base) or saline every consecutive day from P38 to P45, and brain tissue was harvested at P46. Results revealed that neither housing condition reduced nicotine sensitization in NQ rats, whereas enrichment reduced sensitization to nicotine in NS-treated animals. The nucleus accumbens (NAcc) was analyzed for glial cell line-derived neurotrophic factor (GDNF), a neurotrophin important in dopamine plasticity. Results were complex, and revealed that NAcc GDNF was increased in animals given nicotine, regardless of housing condition. Further, enrichment increased GDNF in NQ rats regardless of adolescent drug treatment and in NS-treated rats given nicotine, but did not increase GDNF in NS-treated controls compared to the isolated housing condition. This study demonstrates that environmental experience has a prominent impact on the behavioral and the neural plasticity NAcc response to nicotine in adolescence.” (Brown et al. 2018).

“The insular cortex is known to play a pivotal role in addiction to nicotine. Long-term depression (LTD) in the central nervous system is a major form of synaptic plasticity which is involved in learning and memory and in various pathological conditions such as nicotine addiction. Until now, effects of nicotine on LTD were mainly examined in the hippocampus and striatum, and there is no report showing the effects of nicotine on LTD in the insular cortex. In the present study, I show for the first time that nicotine facilitates LTD which is induced by combination of presynaptic stimulation with postsynaptic depolarization (paired training) in layer 5 pyramidal neurons of the mouse insular cortex using whole-cell patch-clamp recordings. The facilitatory effect of nicotine on LTD was blocked by GABAA receptor antagonists, bicuculline and picrotoxin. Furthermore, blockade of β2-containing nicotinic acetylcholine receptors (nAChRs) prevented the effects of nicotine on LTD. Taken together, these results suggest that in layer 5 pyramidal neurons of the insular cortex, nicotine facilitates LTD through enhancement of GABAergic synaptic transmission, presumably mediated by activation of β2-containing nAChRs. These findings may provide the crucial synaptic basis for the insular cortical changes in nicotine addiction.” (Toyoda 2018).

“Nicotine is the major neurotoxicant in cigarettes that affects many transmitter systems within the brain as well as other factors, including the growth factors. Brain derived neurotrophic factor (BDNF), is the most abundant growth factor in the brain and plays a critical role in early new neuron differentiation, development and synapsis growth, and the survival of fully developed neurons and synaptic activity. Over the past 3 decades, data has emerged on the effects of nicotine and cigarette smoke exposure on the expression of BDNF and its primary specific receptor tyrosine kinase receptor B (TrkB). This review summarizes data regarding the changes in brain BDNF expression after nicotine or cigarette smoke exposure, and discusses their implications considering BDNF's functional roles.” (Machaalani and Chen 2018).

“Pregnant smoking women are frequently episodic drinkers. Here, we investigated whether ethanol exposure restricted to the brain growth spurt period when combined with chronic developmental exposure to nicotine aggravates memory/learning deficits and hyperactivity, and associated cAMP and cGMP signaling disruption. To further investigate the role of these signaling cascades, we verified whether vinpocetine (a phosphodiesterase inhibitor) ameliorates the neurochemical and behavioral outcomes. Swiss mice had free access to nicotine (NIC, 50 μg/ml) or water to drink during gestation and until the 8th postnatal day (PN8). Ethanol (ETOH, 5 g/kg, i.p.) or saline were injected in the pups every other day from PN2 to PN8. At PN30, animals either received vinpocetine (20 mg/kg, i.p.) or vehicle before being tested in the step-down passive avoidance or open field. Memory/learning was impaired in NIC, ETOH and NIC + ETOH mice, and vinpocetine mitigated ETOH- and NIC + ETOH-induced deficits. Locomotor hyperactivity identified in ETOH and NIC + ETOH mice was ameliorated by vinpocetine. While cyclic nucleotides levels in cerebral cortex and hippocampus were reduced by NIC, ETOH and NIC + ETOH, this outcome was more consistent in the latter group. As observed for behavior, vinpocetine normalized NIC + ETOH nucleotides levels. pCREB levels were also increased in response to vinpocetine, with stronger effects in the NIC + ETOH group. Exposure to both drugs of abuse worsens behavioral and neurochemical disruption. These findings and the amelioration of deleterious effects by vinpocetine support the idea that cAMP and cGMP signaling contribute to nicotine- and ethanol-induced hyperactivity and memory/learning deficits.” (Abreu-Villaca et al. 2018).

“Nicotine is a major addictive compound in tobacco and a component of smoking-related products, such as e-cigarettes. Once internalized, nicotine can perturb many cellular pathways and can induce alterations in proteins across different cell types; however, the mechanisms thereof remain undetermined. The authors hypothesize that both tissue-specific and global protein abundance alterations result from nicotine exposure. Presented here is the first proteomic profiling of multiple tissues from mice treated orally with nicotine. Proteins extracted from seven tissues (brain, heart, kidney, liver, lung, pancreas, and spleen) from treated (n = 5) and untreated control (n = 5) mice are assembled into a TMT10-plex experiment. A minimalistic proteomics strategy is employed using TMT reagents efficiently and centrifugation-based reversed-phase columns to streamline sample preparation. Combined, over 11 000 non-redundant proteins from over 138 000 different peptides are quantified in seven TMT10-plex experiments. Between 7 and 126 proteins are significantly altered in tissues from nicotine-exposed mice, 11 which are altered in two or more tissues. Our data showcase the vast extent of nicotine exposure across murine tissue.” (Paulo et al. 2018).

“Nicotinic acetylcholine receptors (nAChR), the primary cell surface targets of nicotine, have implications in various neurological disorders. Here we investigate the proteome-wide effects of nicotine on human haploid cell lines (wildtype HAP1 and α7KO-HAP1) to address differences in nicotine-induced protein abundance profiles between these cell lines. We performed an SPS-MS3-based TMT10-plex experiment arranged in a 2-3-2-3 design with two replicates of the untreated samples and three of the treated samples for each cell line. We quantified 8775 proteins across all ten samples, of which several hundred differed significantly in abundance. Comparing α7KO-HAP1 and HAP1wt cell lines to each other revealed significant protein abundance alterations; however, we also measured differences resulting from nicotine treatment in both cell lines. Among proteins with increased abundance levels due to nicotine treatment included those previously identified: APP, APLP2, and ITM2B. The magnitude of these changes was greater in HAP1wt compared to the α7KO-HAP1 cell line, implying a potential role for the α7 nAChR in HAP1 cells. Moreover, the data revealed that membrane proteins and proteins commonly associated with neurons were predominant among those with altered abundance. This study, which is the first TMT-based proteome profiling of HAP1 cells, defines further the effects of nicotine on non-neuronal cellular proteomes.” (Paulo and Gygi 2018).

“The objective of the current study is to test the hypothesis that the deletion of alpha(α)2\* nicotinic acetylcholine receptors (nAChRs) (encoded by the Chrna2 gene) ablate maternal nicotine-induced learning and memory deficits in adolescent mice. We use a pre-exposure-dependent contextual fear conditioning behavioral paradigm that is highly hippocampus-dependent. Adolescent wild type and α2-null mutant offspring are exposed to vehicle or maternal nicotine exposure (200 μg/ml, expressed as base) in the drinking water throughout pregnancy until weaning. Adolescent male offspring mice are tested for alterations in growth and development characteristics as well as modifications in locomotion, anxiety, shock-reactivity and learning and memory. As expected, maternal nicotine exposure has no effects on pup number, weight gain and only modestly reduces fluid intake by 19%. Behaviorally, maternal nicotine exposure impedes extinction learning in adolescent wild type mice, a consequence that is abolished in α2-null mutant mice. The effects on learning and memory are not confounded by alternations in stereotypy, locomotion, anxiety or sensory shock reactivity. Overall, the findings highlight that the deletion of α2\* nAChRs eliminate the effects of maternal nicotine exposure on learning and memory in adolescent mice.” (Mojica et al. 2018).

“Despite dissemination of information regarding the harm on fetal development of smoking while pregnant, the number of pregnancies associated with nicotine exposure appears to have stagnated. Presence of nicotine during neural formulation is associated with a higher susceptibility of drug dependence, suggesting an altered development of neurons in circuits involved in saliency and motivation. The laterodorsal tegmental nucleus (LDT) plays a role in coding stimuli valence via afferents to mesolimbic nuclei. Accordingly, alterations in development of neural mechanisms in the LDT could be involved in vulnerability to drug dependency. Therefore, we examined the effect of prenatal nicotine exposure (PNE) on glutamatergic functioning of LDT neurons in mouse brain slices using whole-cell, patch clamp concurrent with fluorescence-based calcium imaging. PNE was associated with larger amplitudes of AMPA-induced currents, and greater AMPA-mediated rises in intracellular calcium. AMPA/NMDA ratios and the AMPA-current rectification index were lower and higher, respectively, consistent with changes in the functionality of AMPA receptors in the PNE, which was substantiated by a greater inhibition of evoked and spontaneous glutamatergic synaptic events by a selective inhibitor of GluA2-lacking AMPA receptors. Paired pulse ratios showed a decreased probability of glutamate release from presynaptic inputs, and fluorescent imaging indicated a decreased action potential-dependent calcium increase associated with PNE. When taken together, our data suggest that PNE alters LDT glutamatergic functioning, which could alter output to mesolimbic targets. Such an alteration could play a role in altered coding of relevancy of drug stimuli that could enhance risk for development of drug dependency.” (Polli and Kohlmeier 2018).

“Numerous studies have attributed the psychopathology of anxiety and stress disorders to maladaptive behavioral responses such as an inability to extinguish fear. Therefore, understanding neural substrates of fear extinction is imperative for developing more effective therapies for anxiety and stress disorders. Although several studies indicated a role for cholinergic transmission and nicotinic acetylcholine receptors (nAChRs) in anxiety and stress disorder symptomatology, very little is known about the specific contribution of nAChRs in the fear extinction process. In the present study, we first examined the involvement of several brain regions essential for fear extinction (i.e., dorsal and ventral hippocampus, dHPC and vHPC; infralimbic, IL, and prelimbic, PL of the medial prefrontal cortex, mPFC; basolateral nucleus of the amygdala, BLA) in the impairing effects of a nAChR agonist, nicotine, on contextual fear extinction in mice. Our results showed that systemic administration of nicotine during contextual fear extinction increased c-fos expression in the vHPC and BLA while not affecting dHPC, IL or PL. In line with these results, local nicotine infusions into the vHPC, but not dHPC, resulted in impaired contextual fear extinction. Interestingly, we found that local nicotine infusions into the PL also resulted in impairment of contextual fear extinction. Second, we measured the protein levels of the GABA synthesizing enzymes GAD65 and GAD67 in the dHPC and vHPC during contextual fear extinction. Our results showed that in the group that received acute nicotine, both GAD65 and GAD67 protein levels were downregulated in the vHPC, but not in dHPC. This effect was negatively correlated with the level of freezing response during fear extinction suggesting that the downregulated GAD65/67 levels were associated with disrupted fear extinction. Finally, using c-fos/GAD65/67 double immunofluorescence, we showed that nicotine mainly increased c-fos expression in non-GABAergic ventral hippocampal cells, indicating that acute nicotine increases vHPC excitability. Overall, our results suggest that acute nicotine's impairing effects on fear extinction are associated with ventral hippocampal disinhibition. Therefore, these results further our understanding of the interaction between nicotine addiction and anxiety and stress disorders by describing novel neural mechanisms mediating fear extinction.” (Kutlu et al. 2018).

“Nicotine, an addictive substance, is absorbed from the lungs following inhalation of tobacco smoke, and distributed to various tissues such as liver, brain, and retina. Recent in vivo and in vitro studies suggest the involvement of a carrier-mediated transport process in nicotine transport in the lung, liver, and inner blood-retinal barrier. In addition, in vivo studies of influx and efflux transport of nicotine across the blood-brain barrier (BBB) revealed that blood-to-brain influx transport of nicotine is more dominant than brain-to-blood efflux transport of nicotine. Uptake studies in TR-BBB13 cells, which are an in vitro model cell line of the BBB, suggest the involvement of H+/organic cation antiporter, which is distinct from typical organic cation transporters, in nicotine transport at the BBB. Moreover, inhibition studies in TR-BBB13 cells showed that nicotine uptake was significantly reduced by central nervous system (CNS) drugs, such as antidepressants, anti-Alzheimer's disease drugs, and anti-Parkinson's disease drugs, suggesting that the nicotine transport system can recognize these molecules. The cumulative evidence would be helpful to improve our understanding of smoking-CNS drug interaction for providing appropriate medication.” (Tega et al. 2018).

“Nicotine promotes interoceptive changes in the nervous system. Such interoceptive stimuli play important roles in modulating addictive behavior. Operant and Pavlovian stimulus control modulate responsiveness to environmental stimuli related to drug-seeking and self-administration. Nicotine functions as a discriminative stimulus in modulating operant behavior as well as Pavlovian feature stimuli in modulating the conditional responding (CR) to exteroceptive CS→US contingencies. Elucidation of the interaction of these interoceptive stimulus control functions is vital for a comprehensive understanding of nicotine use/abuse, which might lead to better behavioral treatment strategies. This experiment evaluated the interaction among Pavlovian feature positive (FP) and feature negative (FN) effects of nicotine on concurrently occurring operant SD and SΔ effects. Sixteen rats were trained in a Pavlovian and operant bidirectional contingency paradigm, using nicotine (0.3 mg/kg) and non-drug (saline) states as interoceptive cues for operant discriminative stimulus conditions (SD and SΔ) as well as Pavlovian FP and FN for a light-CS, either leading to a shared food pellet outcome or non-outcome. Nicotine and saline sessions were intermixed. For one group of rats (n = 8), nicotine served as an SD for lever pressing (variable interval 60 s) and simultaneously functioned as an FN for CS-light→noUS relation on the same sessions. On intermixed sessions, saline served as the SΔ for lever pressing (non-reinforced) and FP, during which the 8-sec light preceded delivery of the food pellet (variable time ITI = 60 s). For the other group (n = 8) nicotine served as the SΔ (lever pressing non-reinforced) and FP for the CS, with saline serving in the reverse roles. Consecutive brief non-reinforcement tests revealed that: A) rates of lever pressing were significantly greater in SD than SΔ with nicotine and saline suggesting strong operant discriminative stimulus control; B) FP responding to the light CS with nicotine and saline was evident; and C) FN suppression of the CR with nicotine was not evident but weak under saline. These data suggest that nicotine can function as an interoceptive context that hierarchically can enter into concurrently opposing modulatory relations in Pavlovian and operant drug discrimination procedures.” (Troisi and Michaud 2019)

“Nicotinic acetylcholine receptors are cationic channels that mediate fast excitatory transmission in the central nervous system. Several nicotinic acetylcholine receptor subunits have been detected within cerebellar granule cell layer (GCL), and activation of these receptors may have a significant influence on neuronal synaptic transmission of the cerebellum. The aim of present study was to better understand the roles of nicotinic acetylcholine receptors during the sensory stimulation-evoked synaptic transmission in the cerebellar GCL. Our results showed that cerebellar surface perfusion of nicotine significantly facilitated the cerebellar GCL field potential responses evoked by air-puff stimulation of ipsilateral whisker pad, which exhibited increases in amplitude and area under the curve (AUC) of both stimulus onset responses (N1) and stimulus offset responses (N2). The nicotine-induced increase in AUC of facial stimulation-evoked N1 was dose-dependent with a 50% effective concentration (EC50) of 32.6 μM. Application of either a selective α4β2 nicotinic acetylcholine receptors antagonist, DHβE (1 μM) or a selective α7 nicotinic acetylcholine receptors antagonist, MLA (1 μM) alone attenuated, but not completely abolished the nicotine-induced increases in the amplitude and AUC of the facial stimulation-evoked N1. However, simultaneous blockade of α7 and α4β2 nicotinic acetylcholine receptor subunits abolished the nicotine-induced increase in the amplitude of N1. These results indicate that nicotine activates α7 and α4β2 nicotinic acetylcholine receptor subunits, resulting in an enhancement of facial stimulation-evoked responses in mouse cerebellar GCL. Our results suggest that nicotine modulates the sensory information processing in the cerebellar GCL through α7 and α4β2 subunits nicotinic acetylcholine receptors.” (Xu H et al. 2019).

“Dopamine (DA) neurons in the ventral tegmental area (VTA) are thought to encode reward prediction errors (RPE) by comparing actual and expected rewards. In recent years, much work has been done to identify how the brain uses and computes this signal. While several lines of evidence suggest the interplay of the DA and the inhibitory interneurons in the VTA implements the RPE computation, it still remains unclear how the DA neurons learn key quantities, for example the amplitude and the timing of primary rewards during conditioning tasks. Furthermore, endogenous acetylcholine and exogenous nicotine, also likely affect these computations by acting on both VTA DA and GABA (γ -aminobutyric acid) neurons via nicotinic-acetylcholine receptors (nAChRs). To explore the potential circuit-level mechanisms for RPE computations during classical-conditioning tasks, we developed a minimal computational model of the VTA circuitry. The model was designed to account for several reward-related properties of VTA afferents and recent findings on VTA GABA neuron dynamics during conditioning. With our minimal model, we showed that the RPE can be learned by a two-speed process computing reward timing and magnitude. By including models of nAChR-mediated currents in the VTA DA-GABA circuit, we showed that nicotine should reduce the acetylcholine action on the VTA GABA neurons by receptor desensitization and potentially boost DA responses to reward-related signals in a non-trivial manner. Together, our results delineate the mechanisms by which RPE are computed in the brain, and suggest a hypothesis on nicotine-mediated effects on reward-related perception and decision-making.” (Deperrois et al. 2018).

“Prenatal nicotine exposure (PNE) is closely related to depression in offspring. However, the underlying mechanism is still unclear. We hypothesized that neurosteroid in the hippocampus may mediate PNE‑induced depression‑like behaviors. Nicotine was subcutaneously administered (1.0 mg/kg) to pregnant rats twice daily from gestational day (GD) 9 to 20. In adolescent offspring, PNE significantly increased immobility time and decreased the sucrose preference in female rats. The numbers of hippocampal neurons declined in the CA3 and DG regions. Steroidogenic acute regulatory protein (StAR) expression was suppressed in female rats. In fetal offspring, the neuronal numbers of CA3 regions in PNE female fetal hippocampal were significantly decreased, accompanied by the enhanced content of corticosterone and StAR expression. These data indicated that PNE induced depression‑like behavior in adolescent female rats via the regulation of neurosteroid levels in the hippocampus.” (Zhang C et al. 2019).

“INTRODUCTION: Nicotine is an alkaloid that affects the functioning of the central nervous system and produces dependence. In low doses, it acts as a stimulant and relaxant. Nicotine was reported to have pro-cognitive effects in humans and animals. However, high doses of nicotine are harmful for many organs.The aim of the study was to check whether a 30-day exposure to transdermal nicotine affects memory and biochemical parameters in mice. MATERIAL AND METHODS: A total of 32 mice (16 males and 16 females) were used in the experiment. Mice were divided into 4 groups of 8 animals each: I control-females receiving placebo patches for 30 days, II females receiving nicotine patches for 30 days, III control-males receiving placebo patches, IV males receiving nicotine patches. Spontaneous alternation and locomotor activity were examined weekly in a Y-maze. Body mass was recorded daily. On day 30, venous blood samples were obtained and the animals were anaesthetized with CO<sub>2</sub>. Their blood was used to measure alanine transaminase (ALT), asparagine transaminase (AST), cholesterol, creatinine and glycosylated haemoglobin (HbA<sub>1</sub>C). RESULTS: Nicotine significantly improved memory in male mice on day 8. It increased ALT and AST activities in males and females, as well as the concentration of cholesterol in their blood sera. CONCLUSIONS: In conclusion, transdermal nicotine may produce transient improvement in fresh spatial memory in male mice, but it is not a long-term effect and therefore nicotine does not seem to be appropriate for use in the treatment of neurodegenerative disorders. It elevates blood cholesterol level and thus may increase the risk of atherosclerosis and cardiovascular events; moreover, it negatively affects liver enzymes. Nicotine use is therefore not recommended.” (Nieradko-Iwanicka et al. 2019).

“Common variation in the CYP2B6 gene, encoding the cytochrome P450 2B6 enzyme, is associated with substrate-specific altered clearance of multiple drugs. CYP2B6 is a minor contributor to hepatic nicotine metabolism, but the enzyme has been proposed as relevant to nicotine-related behaviors because of reported CYP2B6 mRNA expression in human brain tissue. Therefore, we hypothesized that CYP2B6 variants would be associated with altered nicotine oxidation, and that nicotine metabolism by CYP2B6 would be detected in human brain microsomes. We generated recombinant enzymes in insect cells corresponding to nine common CYP2B6 haplotypes and demonstrate genetically determined differences in nicotine oxidation to nicotine iminium ion and nornicotine for both (S) and (R)-nicotine. Notably, the CYP2B6.6 and CYP2B6.9 variants demonstrated lower intrinsic clearance relative to the reference enzyme, CYP2B6.1. In the presence of human brain microsomes, along with nicotine-N-oxidation, we also detect nicotine oxidation to nicotine iminium ion. However, unlike N-oxidation, this activity is NADPH independent, does not follow Michaelis-Menten kinetics, and is not inhibited by NADP or carbon monoxide. Furthermore, metabolism of common CYP2B6 probe substrates, methadone and ketamine, is not detected in the presence of brain microsomes. We conclude that CYP2B6 metabolizes nicotine stereoselectively and common CYP2B6 variants differ in nicotine metabolism activity, but did not find evidence of CYP2B6 activity in human brain.” (Bloom et al. 2019).

“Maternal smoking has negative long-term consequences on affective behaviors, and in rodents, chronic neonatal nicotine exposure (CNN) results in increased anxiety. In rat pups, acute nicotine stimulation activates brain regions associated with stress and anxiety, but chronic nicotine exposure could desensitize of nicotinic acetylcholine receptors, the molecular target of nicotine. Here, we determined whether CNN affected neuronal activation by an acute nicotine challenge. Using in situ hybridization, we analyzed mRNA expression of the immediate-early genes (IEGs) c-Fos, Arc, Egr-1 and Npas4, which are markers for neuronal activation and implicated in synaptic plasticity. Following CNN (6 mg/kg/day) or control treatment from postnatal day (P)1 to P7, an acute i.p. nicotine (0.7 mg/kg) or saline injection (control) was administered on P8, and brains collected after 30 min. In drug-naive pups, acute nicotine stimulated IEGs expression specifically in brain areas associated with innate anxiety including the paraventricular hypothalamic nucleus, central nucleus of the amygdala (CeA), and locus coeruleus (LC). Following CNN, acute nicotine stimulated IEG expression in all three areas, but activation was significantly reduced in the LC (c-Fos, Egr-1, Npas4), and CeA (c-Fos). Notably, nicotine-induced Npas4 expression was greatly diminished in the LC, which may affect inhibitory synapse formation in noradrenergic neurons. Thus, after CNN, neurons located in areas associated with anxiety brain circuitry maintained responsiveness to nicotine, but tolerance differentially developed to nicotine. In the developing brain, repeated activation by nicotine of areas related to limbic pathways could alter circuit connectivity and increase responsiveness to stress and anxiety later in life.” (Halawa et al. 2018).

“Cognitive impairment in HIV-1 infection is associated with the induction of chronic proinflammatory responses in the brains of infected individuals. The risk of HIV-related cognitive impairment is increased by cigarette smoking, which induces brain inflammation in rodent models. To better understand the role of smoking and the associated immune response on behavioral and motor function in HIV infection, wild-type F344 and HIV-1 transgenic (HIV1Tg) rats were exposed to either smoke from nicotine-containing (regular) cigarettes, smoke from nicotine-free cigarettes, or to nicotine alone. The animals were then tested using the rotarod test (RRT), the novel object recognition test (NORT), and the open field test (OFT). Subsequently, brain frontal cortex from the rats was analyzed for levels of TNF-α, IL-1, and IL-6. On the RRT, impairment was noted for F344 rats exposed to either nicotine-free cigarette smoke or nicotine alone and for F344 and HIV1Tg rats exposed to regular cigarette smoke. Effects from the exposures on the OFT were seen only for HIV1Tg rats, for which function was worse following exposure to regular cigarette smoke as compared to exposure to nicotine alone. Expression levels for all three cytokines were overall higher for HIV1Tg than for F344 rats. For HIV1Tg rats, TNF-α, IL-1, and IL-6 gene expression levels for all exposure groups were higher than for control rats. All F344 rat exposure groups also showed significantly increased TNF-α expression levels. However, for F344 rats, IL-1 expression levels were higher only for rats exposed to nicotine-free and nicotine-containing CS, and no increase in IL-6 gene expression was noted with any of the exposures as compared to controls. These studies, therefore, demonstrate that F344 and HIV1Tg rats show differential behavioral and immune effects from these exposures. These effects may potentially reflect differences in the responsiveness of the various brain regions in the two animal species as well as the result of direct toxicity mediated by the proinflammatory cytokines that are produced by HIV proteins and by other factors that are present in regular cigarette smoke.” (Royal W 3rd et al. 2018).

“Neurochemical alterations associated with behavioral responses induced by re-exposure to nicotine have not been sufficiently characterized in the dorsal striatum. Herein, we report on changes in glutamate concentrations in the rat dorsal striatum associated with behavioral alterations after nicotine challenge. Nicotine challenge (0.4 mg/kg/day, subcutaneous) significantly increased extracellular glutamate concentrations up to the level observed with repeated nicotine administration. This increase occurred in parallel with an increase in behavioral changes in locomotor and rearing activities. In contrast, acute nicotine administration and nicotine withdrawal on days 1 and 6 did not alter glutamate levels or behavioral changes. Blockade of α7 nicotinic acetylcholine receptors (nAChRs) significantly decreased the nicotine challenge-induced increases in extracellular glutamate concentrations and locomotor and rearing activities. These findings suggest that behavioral changes in locomotor and rearing activities after re-exposure to nicotine are closely associated with hyperactivation of the glutamate response by stimulating α7 nAChRs in the rat dorsal striatum.” (Ryu et al. 2017).

“Brain ageing is a complex process which in its pathologic form is associated with learning and memory dysfunction or cognitive impairment. During ageing, changes in cholinergic innervations and reduced acetylcholinergic tonus may trigger a series of molecular pathways participating in oxidative stress, excitotoxicity, amyloid-β toxicity, apoptosis, neuroinflammation, and perturb neurotrophic factors in the brain. Nicotine is an exogenous agonist of nicotinic acetylcholine receptors (nAChRs) and acts as a pharmacological chaperone in the regulation of nAChR expression, potentially intervening in age-related changes in diverse molecular pathways leading to pathology. Although nicotine has therapeutic potential, paradoxical effects have been reported, possibly due to its inverted U-shape dose-response effects or pharmacokinetic factors. Additionally, nicotine administration should result in optimum therapeutic effects without imparting abuse potential or toxicity. Overall, this review aims to compile the previous and most recent data on nicotine and its effects on cognition-related mechanisms and age-related cognitive impairment.” (Majdi et al. 2017).

“Intracerebral hemorrhage (ICH) is associated with diverse sets of neurological symptoms and prognosis, depending on the site of bleeding. Relative rate of hemorrhage occurring in the cerebral cortex (lobar hemorrhage) has been increasing, but there is no report on effective pharmacotherapeutic approaches for cortical hemorrhage either in preclinical or clinical studies. The present study aimed to establish an experimental model of cortical hemorrhage in mice for evaluation of effects of therapeutic drug candidates. Type VII collagenase at 0.015 U, injected into the parietal cortex, induced hemorrhage expanding into the whole layer of the posterior parts of the sensorimotor cortex in male C57BL/6 mice. Mice with ICH under these conditions exhibited significant motor deficits as revealed by beam-walking test. Daily administration of nicotine (1 and 2 mg/kg), with the first injection given at 3 hr after induction of ICH, improved motor performance of mice in a dose-dependent manner, although nicotine did not alter the volume of hematoma. Immunohistochemical examinations revealed that the number of neurons was drastically decreased within the hematoma region. Nicotine at 2 mg/kg partially but significantly increased the number of remaining neurons within the hematoma at 3 days after induction of ICH. ICH also resulted in inflammatory activation of microglia/macrophages in the perihematoma region, and nicotine (1 and 2 mg/kg) significantly attenuated the increase of microglia. These results suggest that nicotine can provide a therapeutic effect on cortical hemorrhage, possibly via its neuroprotective and anti-inflammatory actions.” (Anan et al. 2017).

## *6.4.* *Other organ systems, dependent on the properties of the substance*

“Cigarette smoke is associated to pathological weakening of bone tissue, being considered an important playmaker in conditions such as osteoporosis and periodontal bone loss. In addition, it is also associated with an increased risk of failure in bone regeneration strategies. The present work aimed to characterize the effects of nicotine on human osteoclastogenesis over a hydroxyapatite substrate. Osteoclast precursors were maintained in the absence or presence of the osteoclastogenesis enhancers M-CSF and RANKL, and were further treated with nicotine levels representative of the concentrations observed in the plasma and saliva of smokers. It was observed that nicotine at low concentrations elicit an increase in osteoclast differentiation, but only in the presence of M-CSF and RANKL it was also able to significantly increase the resorbing ability of osteoclasts. A slight downregulation of NFkB pathway and an increase in the production of TNF-α and, particularly PGE2, were involved in the observed effects of nicotine. At high concentrations, nicotine revealed cytotoxic effects, causing a decrease in cell density. In conclusion, nicotine at levels found in the plasma of the smokers, has the ability to act directly on osteoclast precursors, inducing its osteoclastogenic differentiation. The stimulatory behavior appears to be dependent on the stage of osteoclastic differentiation of the precursor cells, which means, in the absence of M-CSF and RANKL, it only favors the initial stages of osteoclast differentiation, while in the presence of the growth factors, a significant increase in their resorbing ability is also achieved.” (Costa-Rodrigues et al. 2018).

“With the increasing popularity of E-cigarettes, chronic exposure to nicotine (NIC) is emerging as a novel risk factor for the kidney. NIC increases oxidative stress in the kidneys, which impairs the viability and function of renal tubular and endothelial cells, alters renal hemodynamics, and compromises overall kidney function. Moreover, long-term NIC exposure increases the risk of development and progression of chronic kidney diseases and may escalate the impact of coexisting morbidities such as obesity-associated renal disease, hypertension, renal transplant status, or the toxicity of various anticancer agents. In this review, we summarize experimental findings describing increased renal risk of chronic NIC exposure and explore therapeutic interventions to alleviate adverse effects of NIC.” (Arany et al 2018b).

“BACKGROUND/AIM: We have previously reported that simvastatin exhibits antioxidant properties via extracellular signal-regulated kinase (ERK)/cAMP-response element binding (CREB) protein-dependent induction of heme oxygenase-1 (HO1) and chronic nicotine exposure inhibits ERK/CREB signaling in renal proximal tubule cells (through p66shc). Herein, whether nicotine dampens simvastatin-dependent HO1 induction was determined. MATERIALS AND METHODS: Renal proximal tubule (NRK52E) cells were pre-treated with 200 μM nicotine for 24 h followed by 10 μM simvastatin. Promoter activity of HO1 and manganese superoxide dismutase (MnSOD) and activation of CREB and ERK (via ELK1) were determined in luciferase reporter assays. CREB and p66shc were modulated via genetic means. RESULTS: Nicotine suppressed simvastatin-dependent activation of HO1 and MnSOD promoters and activity of CREB and ELK1 via p66shc. Overexpression of CREB or knockdown of p66shc restored simvastatin-dependent induction of HO1 and MnSOD in the presence of nicotine. CONCLUSION: Antioxidant efficiency of simvastatin might be significantly lessened in smokers/E-cigarette users.” (Arany et al. 2018a).

“Tobacco and alcohol are often co-abused. Nicotine can enhance alcoholic fatty liver, and CYP2A6 (CYP2A5 in mice), a major metabolism enzyme for nicotine, can be induced by alcohol. CYP2A5 knockout (cyp2a5-/-) mice and their littermates (cyp2a5+/+) were used to test whether CYP2A5 has an effect on nicotine-enhanced alcoholic fatty liver. The results showed that alcoholic fatty liver was enhanced by nicotine in cyp2a5+/+ mice but not in the cyp2a5-/- mice. Combination of ethanol and nicotine increased serum triglyceride in cyp2a5+/+ mice but not in the cyp2a5-/- mice. Cotinine, a major metabolite of nicotine, also enhanced alcoholic fatty liver, which was also observed in cyp2a5+/+ mice but not in the cyp2a5-/- mice. Nitrotyrosine and malondialdehyde (MDA), markers of oxidative/nitrosative stress, were induced by alcohol and were further increased by nicotine and cotinine in cyp2a5+/+ mice but not in the cyp2a5-/- mice. Reactive oxygen species (ROS) production during microsomal metabolism of nicotine and cotinine was increased in microsomes from cyp2a5+/+ mice but not in microsomes from cyp2a5-/- mice. These results suggest that nicotine enhances alcoholic fatty liver in a CYP2A5-dependent manner, which is related to ROS produced during the process of CYP2A5-dependent nicotine metabolism.” (Chen X et al. 2018).

“The aim of this study was to evaluate the effect of acute administration of nicotine and ethanol on tooth movement in rats. Two hundred rats were divided into eight groups: S: saline; N: nicotine; E: ethanol; NE: nicotine and ethanol; SM: saline with tooth movement; NM: nicotine with tooth movement; EM: ethanol with tooth movement; and NEM: nicotine and ethanol with tooth movement. All the solutions were applied for 32, 44, or 58 days, according to the subgroup. Orthodontic movement (25 cN) was initiated 30 days after solution administration in the groups with tooth movement. The rats were euthanized 2, 14, or 28 days after initiation of tooth movement. Tooth sections were stained using picrosirius and tartrate-resistant acid phosphatase (TRAP). The data were compared by ANOVA using Tukey's HSD and Games-Howell. On day 28 of tooth movement, the NEM group had a lower percentage of type I collagen compared to the SM group (p = 0.0448), and the S group had a higher number of osteoclasts/μm2 compared to the N group (p = 0.0405). Nicotine and ethanol did not affect the tooth movement rate, regardless of induction of orthodontic movement. Nicotine influenced the number of osteoclasts by decreasing their quantity when dental movement was not induced. When nicotine was associated with ethanol, it interfered in the maturation of collagen fibers during orthodontic movement.” (Araujo et al. 2018).

“Although the risks of smoking are well known, the effects of exposure to nicotine on endocrine functions remain unclear. We investigated the deleterious effects of nicotine on the adrenal gland and the mechanisms of these changes in rats. The role of melatonin in ameliorating pathological changes also was investigated. We used 24 rats divided into four groups of six: group 1, control; group 2, nicotine treated; group 3, nicotine and melatonin treated; group 4, melatonin treated. We used histology; immunohistochemistry of inducible nitric oxide synthase (iNOS), vascular endothelial growth factor (VEGF) and tyrosine hydroxylase (TH); measured oxidative and antioxidative markers, malondialdehyde (MDA) and glutathione (GSH); and performed real-time PCR for NF-κB 65, IL1-B and IL6. We also performed histomorphometric analysis. Indentation and lamellar separation of the adrenal capsule, vacuolated degenerated cells and lymphocytic infiltration were observed in group 2. Vacuolated cells and cells with pyknotic nuclei also were detected in the zona reticularis and medulla of the same group. We observed improved shape and cellular lining of the gland in groups 3 and 4. Widespread expression of iNOS, VEGF and TH, increased area percent collagen, decreased GSH (56%) and increased MDA, NF-κB, IL1-B and IL-6 were observed in group 2. All parameters were ameliorated in groups 3 and 4. The effects of nicotine on the adrenal gland can be attributed to oxidative and inflammatory stress; melatonin ameliorates these effects.” (Abdel Fattah et al. 2019).

“PURPOSE: To build a murine model for tobacco smoke and electronic cigarette vapor exposure to characterize the inflammatory and immune responses in the larynx. MATERIALS AND METHODS: In this pilot study, twenty-four wild-type C57BL/6 mice were divided into four groups: smoke, vapor with nicotine, vapor without nicotine, and air only. Following daily exposure for 4 months, larynges were dissected and processed with cytokine detection arrays. Each laryngeal cytokine level between the four different groups was analyzed statistically by using statistical analysis software (SAS) to calculate the analysis of variance (ANOVA). RESULTS: IL-4 was the only cytokine found to achieve statistically significant different levels in this study, with elevated levels of IL-4 in the tobacco smoke and vapor with nicotine groups compared to the levels found in the vapor without nicotine and air only groups (p = 0.0418). While statistically non-significant, prominent findings revealed up-regulation of TGF-β2 and TGF-β3 in the smoke group, but near-normal levels of TGF-β2 and TGF-β3 and suppression of IL-10 in the vapor groups (p > 0.05). CONCLUSION: The potential utility of the murine model is established for studying the inflammatory and immune effects of tobacco smoke and vapor on the mammalian larynx. IL-4 levels in mice larynges were significantly elevated in the tobacco smoke and vapor with nicotine groups.” (Ha et al. 2019).

“Cigarette smoking has been identified as a major risk factor for osteoporosis decades ago. Several studies have shown a direct relationship between cigarette smoking, decreased bone mineral density, and impaired fracture healing. However, the mechanisms behind impaired fracture healing and cigarette smoking are yet to be elucidated. Migration and osteogenesis of mesenchymal stem/stromal cells (MSCs) into the fracture site play a vital role in the process of fracture healing. In human nicotine, the most pharmacologically active and major addictive component present in tobacco gets rapidly metabolized to the more stable cotinine. This study demonstrates that physiological concentrations of both nicotine and cotinine do not affect the osteogenic differentiation of MSCs. However, cigarette smoke exposure induces oxidative stress by increasing superoxide radicals and reducing intracellular glutathione in MSCs, negatively affecting osteogenic differentiation. Although, not actively producing reactive oxygen species (ROS) nicotine and cotinine inhibit catalase and glutathione reductase activity, contributing to an accumulation of ROS by cigarette smoke exposure. Coincubation with N-acetylcysteine or L-ascorbate improves impaired osteogenesis caused by cigarette smoke exposure by both activation of nuclear factor erythroid 2-related factor 2 (Nrf2) signaling and scavenging of ROS, which thus might represent therapeutic targets to support fracture healing in smokers.” (Aspera-Werz et al. 2018).

“The present study examined the effects of nicotinic acetylcholine receptor activation on the odor-induced blood flow response in the olfactory bulb. In urethane-anesthetized rats, odor stimulation (5% amyl acetate, 30 s) produced an increase in olfactory bulb blood flow (reaching 107% ± 3% of the pre-stimulus basal values), without changes in frontal cortical blood flow or mean arterial pressure. Intravenous injection of nicotine (30 μg/kg), a nicotinic acetylcholine receptor agonist, significantly augmented the odor-induced increase response of olfactory bulb blood flow, without changes in the basal blood flow level. The nicotine-induced augmentation of the olfactory bulb blood flow response to odor was negated by dihydro-β-erythroidine, an α4β2-preferring nicotinic acetylcholine receptor antagonist. Our results suggest that the activation of α4β2-like neuronal nicotinic acetylcholine receptors in the brain potentiates an odor-induced blood flow response in the olfactory bulb.” (Uchida et al. 2019).

“Given clear evidence that smoking lowers weight, it is possible that individuals with higher body mass index (BMI) smoke in order to lose or maintain their weight. We performed Mendelian randomization (MR) analyses of the effects of BMI on smoking behaviour in UK Biobank and the Tobacco and Genetics Consortium genome-wide association study (GWAS), on cotinine levels and nicotine metabolite ratio (NMR) in published GWAS and on DNA methylation in the Avon Longitudinal Study of Parents and Children. Our results indicate that higher BMI causally influences lifetime smoking, smoking initiation, smoking heaviness and also DNA methylation at the aryl-hydrocarbon receptor repressor (AHRR) locus, but we do not see evidence for an effect on smoking cessation. While there is no strong evidence that BMI causally influences cotinine levels, suggestive evidence for a negative causal influence on NMR may explain this. There is a causal effect of BMI on smoking, but the relationship is likely to be complex due to opposing effects on behaviour and metabolism.” (Taylor et al. 2019).

“Clinical study showed that smoking during pregnancy deceased the thymus size in newborns. However, the long-term effect remains unclear. This study was aimed to observe the effects of prenatal nicotine exposure (PNE) on the development of thymus and the T-lymphocyte subpopulation in mice offspring from the neonatal to adulthood. Both the thymus weight and cytometry data indicated that PNE caused persistent thymic hypoplasia in male offspring from neonatal to adult period and transient changes in female offspring from neonatal to prepuberal period. Flow cytometry analysis disclosed a permanent decreased proportion and number of mature CD4 single-positive (SP) T cells in thymus of both sex. In addition, the PNE male offspring showed a more serious thymus atrophy in the ovalbumin (OVA)-sensitized model. Moreover, increased autophagic vacuole and elevated mRNA expression of Beclin 1 were noted in PNE fetal thymus. In conclusion, PNE offspring showed thymus atrophy and CD 4 SP T cell reduction at different life stages. Mechanically, PNE induced excessive autophagy in fetal thymocytes might be involved in these changes. All the results provided evidence for elucidating the PNE-induced programmed immune diseases.” (Qu et al. 2019).

“Long term exposure to oral smokeless tobacco may induce lesions in the oral cavity characterized by a hyperplastic epithelium. The possible role of nicotine and the physical properties of oral tobacco for developing these lesions, as well as of dysplasia and neoplasia is unclear. Low nitrosamine Swedish snus as well as non-genotoxic butylated hydroxyanisole induces increased cellular proliferation in the rat forestomach epithelia. Using this model, we report here on the effects of nicotine, pH, and particle size. Snus with different properties had no impact on oxidative stress as determined by 8-oxo-7,8-dihydro-2'-deoxyguanosine, or on interleukin IL-1b. Whereas BHA boosted IL-6, probably due to the presence of nicotine. there was no significant enhancement of cell divisions with increasing particle size, although in individual samples the variations in proliferation rates increased greatly with increasing particle size. Conforming to human experience, the enhanced cell proliferation caused by snus was found to be completely reversible. A cacao bean extract had a protective action similar to that previously found for blueberries. The main cause of the observed tobacco induced cell proliferation could be mechanical irritation, possibly in combination with nicotine, whereas within the studied range, pH did not affect the rate of cell division.” (Joksić et al. 2019).

“BACKGROUND: Nicotine is associated with increased incidence of periodontal disease and poor response to therapy. This article aimed at identifying the expression of matrix metalloproteinases 2 (MMPs2) and vascular endothelial growth factor (VEGF) proteins on extracellular matrix, fibrous distribution and angiogenetic development in periodontitis caused by nicotine effects on periodontal membrane. MATERIALS AND METHODS: In this experimental study, rats were divided into nicotine and control groups. While the rats in the nicotine group (n = 6) were administered 2 mg/kg nicotine sulphate for 28 days, the animals in the control group (n = 6) were only administered 1.5 mL physiologic saline solution subcutaneously for 28 days. RESULTS: Histological sections were prepared and immunohistochemically stained for MMP2 and VEGF. The sections stained with Trichrome-Masson were observed under light microscope. VEGF and MMP2 immunoreactivity of periodontal gingiva and dentin was assessed by immunohistochemical staining. CONCLUSIONS: Nicotine reduces MMP production, disrupts collagen synthesis and causes periodontitis. We observed that nicotine increases periodontitis by disrupting periodontal membrane and prevents tooth to anchor in dental alveoli by disrupting epithelial structure.” (Deveci et al. 2018).

“Nicotine contained in cigarette smoke contributes to the onset of several diseases, including osteoporosis, whose emerging pathogenic mechanism is associated with osteoblasts apoptosis. Scanty information is available on the molecular mechanisms of nicotine on osteoblasts apoptosis and, consequently, on an important aspect of the pathogenesis of smokers-related osteoporosis. Glyoxalase 1 (Glo1) is the detoxification enzyme of methylglyoxal (MG), a major precursor of advanced glycation end products (AGEs), potent pro-apoptotic agents. Hydroimidazolone (MG-H1) is the major AGE derived from the spontaneous MG adduction of arginine residues. The aim of this study was to investigate whether, and by means of which mechanism, the antiglycation defence Glo1 was involved in the apoptosis induced by 0.1 and 1µM nicotine in human primary osteoblasts chronically exposed for 11 and 21 days. By using gene overexpression/silencing and scavenging/inhibitory agents, we demonstrated that nicotine induces a significant intracellular accumulation of hydrogen peroxide (H2O2) that, by inhibiting Glo1, drives MG-H1 accumulation/release. MG-H1, in turn, triggers H2O2 overproduction via receptor for AGEs (RAGE) and, in parallel, an apoptotic mitochondrial pathway by inducing Transglutaminase 2 (TG2) downregulation-dependent NF-kB desensitization. Measurements of H2O2, Glo1 and MG-H1 circulating levels in smokers compared with non-smokers or in smokers with osteoporosis compared with those without this bone-related disease supported the results obtained in vitro. Our findings newly pose the antiglycation enzymatic defense Glo1 and MG-H1 among the molecular events involved in nicotine-induced reactive oxygen species-mediated osteoblasts apoptosis, a crucial event in smoker-related osteoporosis, and suggest novel exposure markers in health surveillance programmes related to smokers-associated osteoporosis.” (Marinucci et al. 2018).

“E-cigarette smoke delivers stimulant nicotine as aerosol without tobacco or the burning process. It contains neither carcinogenic incomplete combustion byproducts nor tobacco nitrosamines, the nicotine nitrosation products. E-cigarettes are promoted as safe and have gained significant popularity. In this study, instead of detecting nitrosamines, we directly measured DNA damage induced by nitrosamines in different organs of E-cigarette smoke-exposed mice. We found mutagenic O6-methyldeoxyguanosines and γ-hydroxy-1,N2 -propano-deoxyguanosines in the lung, bladder, and heart. DNA-repair activity and repair proteins XPC and OGG1/2 are significantly reduced in the lung. We found that nicotine and its metabolite, nicotine-derived nitrosamine ketone, can induce the same effects and enhance mutational susceptibility and tumorigenic transformation of cultured human bronchial epithelial and urothelial cells. These results indicate that nicotine nitrosation occurs in vivo in mice and that E-cigarette smoke is carcinogenic to the murine lung and bladder and harmful to the murine heart. It is therefore possible that E-cigarette smoke may contribute to lung and bladder cancer, as well as heart disease, in humans.” (Lee et al. 2018).

“Electronic (e)-cigarettes theoretically may be safer than conventional tobacco. However, our prior studies demonstrated direct adverse effects of e-cigarette vapor (EV) on airway cells, including decreased viability and function. We hypothesize that repetitive, chronic inhalation of EV will diminish airway barrier function, leading to inflammatory protein release into circulation, creating a systemic inflammatory state, ultimately leading to distant organ injury and dysfunction. C57BL/6 and CD-1 mice underwent nose only EV exposure daily for 3-6 mo, followed by cardiorenal physiological testing. Primary human bronchial epithelial cells were grown at an air-liquid interface and exposed to EV for 15 min daily for 3-5 days before functional testing. Daily inhalation of EV increased circulating proinflammatory and profibrotic proteins in both C57BL/6 and CD-1 mice: the greatest increases observed were in angiopoietin-1 (31-fold) and EGF (25-fold). Proinflammatory responses were recapitulated by daily EV exposures in vitro of human airway epithelium, with EV epithelium secreting higher IL-8 in response to infection (227 vs. 37 pg/ml, respectively; P < 0.05). Chronic EV inhalation in vivo reduced renal filtration by 20% ( P = 0.017). Fibrosis, assessed by Masson's trichrome and Picrosirius red staining, was increased in EV kidneys (1.86-fold, C57BL/6; 3.2-fold, CD-1; P < 0.05), heart (2.75-fold, C57BL/6 mice; P < 0.05), and liver (1.77-fold in CD-1; P < 0.0001). Gene expression changes demonstrated profibrotic pathway activation. EV inhalation altered cardiovascular function, with decreased heart rate ( P < 0.01), and elevated blood pressure ( P = 0.016). These data demonstrate that chronic inhalation of EV may lead to increased inflammation, organ damage, and cardiorenal and hepatic disease.” (Crotty et al. 2018).

“Exposure to nicotine is known to cause adverse effects in many target organs including kidney. Epidemiological studies suggest that nicotine-induced kidney diseases are prevalent worldwide. However, the impact of duration of exposure on the nicotine-induced adverse effects in normal kidney cells and the underlying molecular mechanism is still unclear. Hence, the objective of this study was to evaluate both acute and long-term effects of nicotine in normal human kidney epithelial cells (HK-2). Cells were treated with 1 and 10 µM nicotine for acute and long-term duration. The result of cell viability showed that the acute exposure to 1 µM nicotine has no significant effect on growth. However, the 10 µM nicotine caused significant decrease in the growth of HK-2 cells. The long-term exposure resulted in significantly increased cell growth in both 1 and 10 µM nicotine-treated groups. Analysis of cell cycle and expression of marker genes related to proliferation and apoptosis further confirmed the effects of nicotine. Additionally, the analysis of growth signaling pathway revealed the decreased level of pAKT in cells with acute exposure whereas the increased level of pAKT in long-term nicotine-exposed cells. This suggests that nicotine, through modulating the AKT pathway, controls the duration-dependent effects on the growth of HK-2 cells. In summary, this is the first report showing long-duration exposure to nicotine causes increased proliferation of human kidney epithelial cells through activation of AKT pathway.” (Chang and Singh 2018).

“Tobacco smoking is a major risk factor for human cancers including urinary bladder carcinoma. Cigarette smoke inhalation in mice and orally administered nicotine in rats and mice increased urothelial cell proliferation. Nicotine, a major component of smoke, induced cell proliferation in multiple cell types in vitro. In the present study, the enhancing effects of nicotine on F344 rat bladder carcinogenesis induced by N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) were examined. Nicotine administered in drinking water for 32 weeks following 4 weeks of BBN treatment significantly increased the incidence and number of urothelial carcinomas dose-dependently. Ki67 and pSTAT3 labeling indices and expression of nicotinic acetylcholine receptor alpha 7 (nAChRα7) in non-tumor bladder urothelial lesions were significantly increased by nicotine, but the TUNEL assay for apoptosis showed no increase. In a 4 week study, inhibitors of nicotinic acetylcholine receptor decreased nicotine-induced urothelial simple hyperplasia and Ki67 labeling index in the bladder and kidney pelvis at a single cytotoxic dose of nicotine (40 ppm). Urothelial cytotoxicity with regenerative proliferation was observed by light and scanning electron microscopy. In vitro, nicotine was not cytotoxic to rat or human immortalized urothelial cells (do not express nicotine receptors) below millimolar concentrations, nor in human RT4, T24 or UMUC3 urothelial carcinoma cells (express nicotine receptors). However, nicotine slightly, but statistically significantly, increased cell proliferation at micromolar concentrations in human urothelial carcinoma cells. These data suggest that nicotine enhances urinary bladder carcinogenesis by inducing cytotoxicity with regenerative proliferation. The possible role of direct mitogenesis, involving nAChR and STAT3 signaling and of nicotine receptors requires further investigation at non-cytotoxic doses of nicotine.” (Suzuki et al. 2018).

“PURPOSE: Mesenchymal stem cells (MSCs) express some of the nicotinic receptor subunits and adenosine receptors. The communication between tissue MSCs with neutrophils has been shown in previous studies. The aim of the present study is to determine the role of nicotine or caffeine on MSCs and its effects on neutrophils. METHODS: After the isolation, MSCs were pulsed with LPS (10 ng/ml) for 1 h. Then, MSCs were incubated with different concentrations of caffeine (0.1, 0.5 and 1 mM) and or with different concentrations of nicotine (0.1, 0.5, and 1 μM) for 48 h. Afterwards, the medium was aspirated and the cells were used for co-culture experiment with neutrophil. The obtained data showed that LPS primed MSCs could decrease neutrophil vitality, whereas the treatment of MSCs with nicotine and/or especially a treatment with caffeine reverse this effect. RESULTS: Obtained data showed that when the LPS-primed MSCs were treated with nicotine or caffeine, the vitality of co-cultured neutrophils was significantly increased. The rate of the respiratory burst of neutrophils after co-culture by LPS-primed MSCs was decreased compared to the respiratory burst of neutrophil alone. Nicotine and/or caffeine treatment could reverse this reduction. CONCLUSION: Generally, these findings provide a new insight into understanding the anti-inflammatory and immunomodulatory effects of nicotine and caffeine.” (Abbasi et al. 2018).

“Diabetic nephropathy (DN) is a major complication of diabetes mellitus. Clinic reports indicate cigarette smoking is an independent risk factor for chronic kidney disease including DN; however, the underlying molecular mechanisms are not clear. Recent studies have demonstrated that nicotine, one of the active compounds in cigarette smoke, contributes to the pathogenesis of the cigarette smoking-accelerated chronic kidney disease. One of the characteristics of DN is the expansion of mesangium, a precursor of glomerular sclerosis. In the present study, we examined the involvement of Wnt/β-catenin pathway in nicotine-mediated mesangial cell growth in high glucose milieu. Primary human renal mesangial cells were treated with nicotine in the presence of normal (5 mM) or high glucose (30 mM) followed by evaluation for cell growth. In the presence of normal glucose, nicotine increased both the total cell numbers and Ki-67 positive cell ratio, indicating that nicotine stimulated mesangial cell proliferation. Although high glucose itself also stimulated mesangial cell proliferation, nicotine further enhanced the mitogenic effect of high glucose. Similarly, nicotine increased the expression of Wnts, β-catenin, and fibronectin in normal glucose medium, but further increased mesangial cell expression of these proteins in high glucose milieu. Pharmacological inhibition or genetic knockdown of β-catenin activity or expression with specific inhibitor FH535 or siRNA significantly impaired the nicotine/glucose-stimulated cell proliferation and fibronectin production. We conclude that nicotine may enhance renal mesangial cell proliferation and fibronectin production under high glucose milieus partly through activating Wnt/β-catenin pathway. Our study provides insight into molecular mechanisms involved in DN.” (Lan et al. 2018).

“BACKGROUND: Nicotine has negative effects on tissue repair, little research concerns its effect on the cartilage repair of tissue engineering stem cells. The present study aimed to investigate the effects of nicotine on the bone marrow-derived mesenchymal stem cells' (BMSCs) chondrogenic repair function of cartilage defects and explored the molecular mechanism. METHODS: A cartilage defect model of rat was repaired by BMSC transplantation, and treated with nicotine or saline at 2.0 mg/kg/d in 12 weeks. Nicotine's effect on chondrogenic differentiation was studied by exposing BMSCs to nicotine at 0.1, 1, 10, and 100 μM, and methyllycaconitine (MLA), which is a selective α7-nicotinic acetylcholine receptor (nAChR) inhibitor and si-RNA of nuclear factor of activated T cells 2 (NFATc2), were used to verify the molecular mechanism of nicotine's effect. RESULTS: Data showed that nicotine inhibited cartilage repair function by suppressing SRY-type high-mobility group box 9 (Sox9) in regenerated tissues. Further in vitro study demonstrated that nicotine enhanced intracellular Ca2+ and activity of calcineurin (CaN) through α7-nAChR, increased the nucleic expressions of NFATc2 and the bindings to SOX9 promoter, and thus reduced the acetylation of H3K9 and H3K14 in SOX9 promoter. CONCLUSIONS: Findings from this study demonstrated that nicotine suppressed the chondrogenic differentiation of BMSCs in vivo and in vitro, which offers insight into the risk assessment of cartilage defect repair in a nicotine exposure population and its therapeutic target.” (Tie et al. 2018).

“Smoking is a risk factor associated with bone and oral diseases, particularly periodontitis. Nicotine, the major toxic component of tobacco, is able to affect the quality and quantity of bone. Osteoblasts serve an important role in bone formation. Thus far, the effects of nicotine on metabolism‑associated gene and protein expression in osteoblasts have been controversial and the mechanisms remain unclear. The present study assessed alterations in osteogenic activity by performing a Cell Counting kit‑8 assay to investigate proliferation, Annexin V‑fluorescein isothiocyanate/propidium iodide staining to investigate apoptosis, alizarin red staining to investigate the formation of mineralized nodules, reverse transcription‑quantitative polymerase chain reaction and western blotting to investigate the mRNA and protein levels of collagen I, alkaline phosphatase, bone osteocalcin, bone sialoprotein and osteopontin; and mRNA microarray expression analysis, Kyoto Encyclopedia of Genes and Genomes and Gene Ontology analysis to investigate the whole genome expression profile of Sprague‑Dawley (SD) rat primary osteoblasts following treatment with different concentrations of nicotine. The results demonstrated that nicotine inhibited proliferation, promoted early apoptosis and inhibited mineralized nodule formation in a dose‑dependent manner by regulating alkaline phosphatase activity and the expression of osteoblast metabolism‑associated genes and proteins. According to microarray analysis, several genes associated with bone metabolism and genes in the Hedgehog and Notch signaling pathways were downregulated significantly in nicotine‑treated osteoblasts. The results of the present study indicated that nicotine may serve an inhibitory, dose‑dependent role in SD rat primary osteoblasts that may be caused by the perturbation of genes and signaling pathways associated with bone formation. These results may provide a theoretical basis for future research regarding bone metabolism and targeted treatment of oral diseases associated with smoking.” (Liang et al. 2018).

“The enhanced recruitment of leukocytes to the inflamed colon is a key feature of ulcerative colitis (UC). The gut-specific adhesion molecules involved in leukocyte recruitment have emerged as recent therapeutic targets. Nicotine absorbed from smoking has been reported to work protectively in UC patients. Our hypothesis is that nicotine may suppress the aberrant leukocyte recruitment and colonic inflammation via the suppression of the overexpressed gut-specific adhesion molecules in the inflamed colon. To test this hypothesis, the severity of colitis and the degree of leukocyte recruitment induced by gut-specific adhesion molecules were assessed in dextran sulfate sodium (DSS) colitis mice (C57BL/6J mice treated with 3% DSS) with or without nicotine treatment. We also studied the in vitro changes in the expression of adhesion molecules by using a vascular endothelial cell line. DSS-induced colitis was accompanied by increases in disease activity index (DAI), histological score, recruitment of leukocytes, and the expression of adhesion molecules, mucosal vascular address in cell adhesion molecule-1 (MAdCAM-1) and VCAM-1. Nicotine treatment significantly attenuated MAdCAM-1 expression, leukocyte recruitment, DAI, and histological score. The expression of β7-integrin, the ligand for MAdCAM-1, on leukocytes was not affected by nicotine treatment. In vitro study, the TNF-α-enhanced mRNA expression of MAdCAM-1 was reduced by the coadministration of nicotine in a dose-dependent manner, possibly via nicotinic receptor activation. These results supported our hypothesis that nicotine treatment ameliorated colitis through the suppression of MAdCAM-1 expression on the microvessels in the inflamed colon. Further investigation is warranted on the role of nicotine in the treatment of UC.” (Maruta et al. 2018).

“Nicotine-withdrawal symptoms have been indicated as a possible risk factor for neuropsychiatric events, such as depression and suicide, during use of smoking-cessation drugs. We aimed to investigate whether the results of the metabolomic analysis of the rat brain reflect nicotine-withdrawal symptoms. We also aimed to investigate the relative changes in each metabolite in the brains of rats with nicotine-withdrawal symptoms. We created rats experiencing nicotine-withdrawal symptoms through repeat administration of nicotine followed by a 12-h withdrawal period, and rats recovered from nicotine-withdrawal symptoms followed by an 18-h withdrawal period. We then implemented brain metabolic profiling by combining high-resolution magic-angle spinning 1H-NMR spectroscopy with partial least square discriminant analysis (PLS-DA). We found that metabolic profiling of the brain reflects the state during nicotine-withdrawal symptoms and the state after recovery from nicotine-withdrawal symptoms. Additionally, N-acetylaspartate and glutamate increased and aspartate, γ-aminobutyric acid (GABA), and creatine decreased in the hippocampus of rats experiencing nicotine-withdrawal symptoms. Therefore, it is suggested that neurogenesis and neuronal differentiation could be changed and abnormal energy metabolism could occur in the hippocampus during nicotine-withdrawal symptoms.” (Akimoto et al. 2018).

“The hippocampal formation (HF) plays an important role to facilitate higher order cognitive functions. Cholinergic activation of heteromeric nicotinic acetylcholine receptors (nAChRs) within the HF is critical for the normal development of principal neurons within this brain region. However, previous research investigating the expression and function of heteromeric nAChRs in principal neurons of the HF is limited to males or does not differentiate between the sexes. We used whole-cell electrophysiology to show that principal neurons in the CA1 region of the female mouse HF are excited by heteromeric nAChRs throughout postnatal development, with the greatest response occurring during the first two weeks of postnatal life. Excitability responses to heteromeric nAChR stimulation were also found in principal neurons in the CA3, dentate gyrus, subiculum, and entorhinal cortex layer VI (ECVI) of young postnatal female HF. A direct comparison between male and female mice found that principal neurons in ECVI display greater heteromeric nicotinic passive and active excitability responses in females. This sex difference is likely influenced by the generally more excitable nature of ECVI neurons from female mice, which display a higher resting membrane potential, greater input resistance, and smaller afterhyperpolarization potential of medium duration (mAHP). These findings demonstrate that heteromeric nicotinic excitation of ECVI neurons differs between male and female mice during a period of major circuitry development within the HF, which may have mechanistic implications for known sex differences in the development and function of this cognitive brain region.” (Chung and Bailey 2019).

“Epidermal growth factor (EGF) is overexpressed in many cancers and is associated with worse prognosis. EGF binds to its cell surface receptor (EGFR), which induces EGFR phosphorylation. Phosphorylated EGFR (p‑EGFR) is translocated into the nucleus, which increases cancer cell activity. Nicotine, which is one of the main components of tobacco, is absorbed through pulmonary alveoli and mucosal epithelia in the head and neck region by smoking and moves into the blood. Nicotine in blood binds to nicotinic acetylcholine receptor (nAChR) in the central nervous system and serves a crucial role in tobacco addiction. Although nAChR localization is thought to be limited in the nervous system, nAChR is present in a wide variety of non‑neuronal cells, including cancer cells. Recent studies suggest that nicotine contributes to the metastasis and resistance to anti‑cancer drugs of various cancer cells. However, it remains unknown whether head and neck squamous cell carcinoma (HNSCC) cells can utilize nicotine‑nAChR signaling to metastasize and acquire resistance to anti‑cancer drugs, even though the mucosal epithelia of the head and neck region are the primary sites of exposure to tobacco smoke. To the best of our knowledge, the present study is the first to demonstrate the role of nicotine in metastasis and anti‑EGFR‑therapy resistance of HNSCC. The present findings demonstrated that nicotine increased proliferation, migration, invasion, p‑EGFR nuclear translocation and protein kinase B (Akt) phosphorylation in HNSCC cells. It was also demonstrated that nicotine restored cetuximab‑inhibited proliferation, migration and invasion of HNSCC cells. Finally, an in vivo experiment revealed that nicotine increased lymph node metastasis of xenografted tumors, whereas an nAChR inhibitor suppressed lymph node metastasis and p‑EGFR nuclear localization of xenografted tumors. Taken together, these results demonstrated that nicotine induced nuclear accumulation of p‑EGFR, and activation of Akt signaling. These signaling pathways elevated the activities of HNSCC cells, causing lymph node metastasis and serving a role in cetuximab resistance.” (Shimizu et al. 2019).

“Candida albicans ATCC 14053 and Candida parapsilosis ATCC 22019 hyphal-wall protein 1 (HWP1) are involved in hyphae formation and pathogenesis. The transcriptional agglutinin-like sequence 3 (ALS3) genes in both species are responsible for the development of biofilm and colonization on tooth surfaces. Therefore, we investigated the expression of HWP1 and ALS3 quantitatively in C. albicans and C. parapsilosis and examined the biofilm structure upon exposure to various nicotine concentrations. In vitro, biofilms of Candida species were developed directly on slides using the Lab-Tek Chamber Slide System and visualized by confocal laser scanning microscopy. Quantitative real-time polymerase chain reaction was used to measure HWP1 and ALS3 expression in C. albicans ATCC 14053 and C. parapsilosis ATCC 22019. The results indicated that nicotine multiplied the number of yeast cells and increased the extracellular polysaccharides of Candida species. We also found that 1-2 mg/mL nicotine could enhance the formation of biofilm. The findings also revealed that the expression of HWP1 and ALS3 in Candida species were increased as the nicotine concentration increased. Therefore, nicotine influences the biofilm development of oral-associated C. albicans ATCC 14053 and C. parapsilosis ATCC 22019.” (Gunasegar et al. 2019).

“The nicotinic acetylcholine receptor (nAChR) agonist nicotine and the noradrenaline transporter inhibitor atomoxetine are widely studied substances due to their propensity to alleviate cognitive deficits in psychiatric and neurological patients and their beneficial effects on some aspects of cognitive functions in healthy individuals. However, despite growing evidence of acetylcholine-noradrenaline interactions, there are only very few direct comparisons of the two substances. Here, we investigated the effects of nicotine and atomoxetine on response inhibition in the stop-signal task and we characterised the neural correlates of these effects using blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) at 3T. Nicotine (7 mg dermal patch) and atomoxetine (60 mg per os) were applied to N = 26 young, healthy adults in a double-blind, placebo-controlled, cross-over, within-subjects design. BOLD images were collected during a stop-signal task that controlled for infrequency of stop trials. There were no drug effects on behavioural performance or subjective state measures. However, there was a pronounced upregulation of activation in bilateral prefrontal and left parietal cortex following nicotine during successful compared to unsuccessful stop trials. The effect of nicotine on BOLD during failed stop trials was correlated across individuals with a measure of trait impulsivity. Atomoxetine, however, had no discernible effects on BOLD. We conclude that nicotine effects on brain function during inhibitory control are most pronounced in individuals with higher levels of impulsivity. This finding is compatible with previous evidence of nicotine effects on stop-signal task performance in highly impulsive individuals and implicates the nAChR in the neural basis of impulsivity. (Kasparbauer et al. 2019).

“OBJECTIVE: Green Tobacco Sickness (GTS) is an occupational illness caused by dermal absorption of nicotine from tobacco leaves. It affects thousands of farm workers worldwide. Brazil is the second tobacco producer in the world; despite this, there are few studies on GTS among Brazilian harvesters. This study aimed to determine the prevalence of GTS among a population of tobacco workers from a producing area in northeastern Brazil and investigate whether the occurrence of the disease was influenced by factors such age, gender and smoking status. In addition, it was investigated if there was association between the onset of GTS and genetic polymorphisms in genes that encode some detoxification enzymes. A semi-structured questionnaire was used to collect demographic, behavioral and occupational data from the referred workers. Polymorphisms were tested through the Polymerase Chain Reaction technique. RESULTS: The total prevalence of GTS found was 56.9%, with a significant difference between genders (71.7% for women and 35.3% for men, p < 0.0001). No association was identified between the investigated polymorphisms and GTS. This study confirms the occurrence of GTS among tobacco harvesters in Brazil with high prevalence. The investigation suggests the need to take preventive measures to protect tobacco workers against this disease.” (da Mota et al. 2018).

“Studies in animal models have suggested that nicotine, an agonist of nicotinic acetylcholine receptors, may have the potential to prevent and/or reverse the peripheral neuropathy induced by cancer chemotherapeutic drugs, such as paclitaxel and oxaliplatin. However, a large body of evidence suggests that nicotine may also stimulate lung tumor growth and/or interfere with the effectiveness of cancer chemotherapy. Whereas the reported proliferative effects of nicotine are highly variable, the antagonism of antitumor drug efficacy is more consistent, although this latter effect has been demonstrated primarily in cell culture studies. In contrast, in vitro and in vivo studies from our own laboratory indicate that nicotine fails to enhance the growth of nonsmall cell lung cancer cells or attenuate the effects of chemotherapy (paclitaxel). Given the inconsistencies in the literature, coupled with our own findings, the weight of evidence suggests that caution may be warranted in proposing to use nicotine to mitigate chemotherapy-induced peripheral neuropathy in cancer patients receiving chemotherapy. Conversely, clinical trials could be performed in patients who have completed therapy and are considered to be disease-free to determine whether nicotine, in the form of commercially available patches or gum, is effective in alleviating peripheral neuropathy symptoms.” (Kyte and Gewirtz 2018).

“Exposure to nicotine in smoking contributes to most unexplained male infertility but the mechanisms remain to be fully elucidated. Zinc (Zn) is an essential traceelement in normal growth, development and reproduction. Zinc oxide nanoparticles (ZnONPs) are well-known antioxidants. Therefore, this work was designed toinvestigate the potential ability of ZnONPs to protect testis and epididymis innicotine-treated rats. Forty adult male Wistar albino rats were divided intocontrol group and two experimental groups (treated and supplemented rats). In thetreated group, rats received nicotine at a dose of 1 mg/kg/day orally for30 days. Rats in the supplemented group received ZnONPs (10 mg/kg/day) withnicotine (1 mg/kg/day), orally for the same period. Testicular and epididymalsections were stained with H&E to assess the histological changes. Negrosin-eosinstaining of epididymal sperms was performed to assess their viability andmorphological changes. Serum testosterone, FSH and LH levels were assessed. Also,oxidative stress parameters and semiquantitative real-time PCR for steroidogenic enzymes were measured. Morphometric studies of both organs were statisticallyanalyzed. Mild to severe testicular and epididymal structural changes togetherwith sperm morphological abnormalities were detected in nicotine-treated rats.Biochemical results also showed a decrease in all measured parameters except for an increased malondialdehyde (MDA) level that meant deterioration of theirreproductive function. On the other hand, ZnONP supplementation in the last groupshowed an obvious improvement in all investigated parameters.” (Mohamed and Abdelrahman 2019).

“Acute exposure to nicotinic agonists induces myotoxicity in zebrafish embryos. The main goal of this work was to evaluate the potential myotoxicity of nicotine acetylcholine receptor agonists on adult zebrafish muscle tissue by using nicotine as a model compound. Liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) datasets were processed with different chemometric tools based on the selection of Regions of Interest (ROI) and Multivariate Curve-Resolution (ROI-MCR procedure) Alternating Least Squares (ALS) for the analysis of different exposure experiments. Analysis of Variance Simultaneous Component Analysis (ASCA) of changes on metabolite peak profile areas showed significant nicotine concentration and exposure time-dependent changes, clearly differentiating between exposed and non-exposed samples and between short (2 h) and long exposure times (6 h or 24 h). Most of the changes observed in the concentrations of different metabolites are probably secondary to the observed hyperlocomotion, as they have been also observed in humans after strenuous muscular exercise. The absence of myotoxicity might be related with the reduced calcium permeability of adult muscle-type nicotinic acetylcholine receptors (nAChRs).” (Gomez-Canela et al. 2018).

“The use of nicotine as an insecticide was popular in the 1920s and 1930s when cases of occupational poisoning were reported. Lethal effects are due to a curare-like respiratory arrest. [ACGIH] Symptoms of mild poisoning are nausea and vomiting. The cholinergic syndrome (diarrhea, increased salivation and respiratory secretions, and bradycardia) is seen in more severe poisoning. Seizures and respiratory depression are rare complications of severe poisoning. Green tobacco sickness due to skin absorption of nicotine has been described in workers who handle tobacco.” (Haz-Map, 2017)

“More than 1 billion world's population actively smokes tobacco containing the bioactive component nicotine (NT). The biological role of this molecule is mediated through the activation of nicotinic cholinergic receptors, widely distributed in various human tissues including retinal pigmented epithelium. The long-term assumption of NT contributes to several diseases development such as diabetic retinopathy. The major complication of this pathology is the diabetic macular edema (DME), characterized by macular area thinning and blood-retinal barrier (BRB) breakdown. Retinal hyperglycemic/hypoxic microenvironment represents one of the main factors favoring DME progression by eliciting the hypoxia-inducible factors (HIFs) expression. The latter induce new vessels formation by stimulating cellular secretion of vascular endothelial growth factor (VEGF). The etiology of DME is multifactorial, but little is known about the risk factors linked to cigarette smoking, in particular to nicotine's contribution. In the present study, we have investigated the NT role in a model, in vitro, of DME, by evaluating its effect on outer BRB permeability and HIFs/VEGF expression following exposure to hyperglycemic/hypoxic insult. Our results have demonstrated that this compound alters outer BRB integrity exposed to high glucose and low oxygen pressure microenvironment by upregulating HIF-1α/HIF-2α, VEGF expression and ERK1/2 phosphorylation. These data have suggested that NT may play a negative role in active smokers affected by DME.” (Maugeri et al. 2017)

“Nicotine is a major addictive compound in tobacco and a component of smoking-related products, such as e-cigarettes. Once internalized, nicotine can perturb many cellular pathways and can induce alterations in proteins across different cell types; however, the mechanisms thereof remain undetermined. The authors hypothesize that both tissue-specific and global protein abundance alterations result from nicotine exposure. Presented here is the first proteomic profiling of multiple tissues from mice treated orally with nicotine. Proteins extracted from seven tissues (brain, heart, kidney, liver, lung, pancreas, and spleen) from treated (n = 5) and untreated control (n = 5) mice are assembled into a TMT10-plex experiment. A minimalistic proteomics strategy is employed using TMT reagents efficiently and centrifugation-based reversed-phase columns to streamline sample preparation. Combined, over 11 000 non-redundant proteins from over 138 000 different peptides are quantified in seven TMT10-plex experiments. Between 7 and 126 proteins are significantly altered in tissues from nicotine-exposed mice, 11 which are altered in two or more tissues. Our data showcase the vast extent of nicotine exposure across murine tissue.” (Paulo et al. 2018).

# *7.* *Addiction*

“The insular cortex is known to play a pivotal role in addiction to nicotine. Long-term depression (LTD) in the central nervous system is a major form of synaptic plasticity which is involved in learning and memory and in various pathological conditions such as nicotine addiction. Until now, effects of nicotine on LTD were mainly examined in the hippocampus and striatum, and there is no report showing the effects of nicotine on LTD in the insular cortex. In the present study, I show for the first time that nicotine facilitates LTD which is induced by combination of presynaptic stimulation with postsynaptic depolarization (paired training) in layer 5 pyramidal neurons of the mouse insular cortex using whole-cell patch-clamp recordings. The facilitatory effect of nicotine on LTD was blocked by GABAA receptor antagonists, bicuculline and picrotoxin. Furthermore, blockade of β2-containing nicotinic acetylcholine receptors (nAChRs) prevented the effects of nicotine on LTD. Taken together, these results suggest that in layer 5 pyramidal neurons of the insular cortex, nicotine facilitates LTD through enhancement of GABAergic synaptic transmission, presumably mediated by activation of β2-containing nAChRs. These findings may provide the crucial synaptic basis for the insular cortical changes in nicotine addiction.” (Toyoda 2018).

“Scientific discoveries that impact human health motivate many of us and also provide a rationale for public support. Nicotine use disorder (NUD) remains a significant public health burden affecting over 20% of adults in the United States and over 30% of those with other mental health disorders (Chou et al., 2016). Tobacco use is the primary component of NUD and is the leading cause of preventable morbidity and mortality in the United States, being responsible for approximately one in five of such deaths (more than 480,000 deaths annually; Camenga and Klein, 2016; Centers for Disease Control and Prevention, 2019). In spite of intensive government public information campaigns that have highlighted the health risks associated with nicotine use over the past five decades, a fraction of the population continues to suffer NUD. Moreover, the emergence of alternative nicotine delivery products, for example electronic nicotine delivery systems like vaporizers or electronic cigarettes, provide fresh challenges to reducing NUD (U.S. Department of Health and Human Services, 2014). Why does NUD persist in the face of widespread distribution of overwhelming evidence of its dangers? Clearly, the addictive properties of nicotine are a primary obstacle to reducing its use (Davis et al., 1988). An understanding of the molecular mechanism of nicotine dependence could form the basis for developing new and improved NUD therapeutics. The immediate effects of nicotine administration that users seek are mediated by a variety of nicotinic acetylcholine receptor (nAchR) subunit combinations in the plasma membrane of neurons involved in reward circuity (Koob and Volkow, 2016). In addition, nicotine can act on nicotinic receptors positioned at intracellular locations, which affect cell function by an “inside-out” mechanism. The Lester laboratory at Caltech and their collaborators at Janelia Farm have worked to unravel the components of inside-out nicotinic receptor biology and its role in disease mechanisms including Parkinson’s disease and NUD. In this issue of the Journal of General Physiology, Shivange et al. describe the development and characterization of a novel optical nicotine sensor iNicSnFR, which also detects varenicline (Chantix)—a drug that acts on nicotinic receptors and is used as a smoking cessation aid. iNicSnFR affords a means to answer key questions concerning the levels and timing of nicotine and drug exposure at intracellular and extracellular sites, as well as providing a basis for mechanistic investigations of NUD and a possible path to improved therapeutics.” (McManus et al. 2019).

“Acute tolerance to effects of nicotine plays an important role in nicotine dependence, but the mechanism underlying these effects is unclear. Drug discrimination was used in the current study to examine the impact of nicotine pretreatment on sensitivity to the discriminative stimulus effects of nicotine and the FDA-approved smoking cessation pharmacotherapy varenicline. Rhesus monkeys (n = 4) discriminated 0.032 mg/kg nicotine base iv from saline under an FR5 schedule of stimulus-shock termination. Both nicotine and varenicline increased drug-appropriate responding; ED50 values (95% confidence limits) were 0.0087 [0.0025, 0.030] and 0.028 [0.0096, 0.082] mg/kg, respectively. Additional pretreatment injections of the training dose of nicotine (0.032 mg/kg, iv) produced tolerance to its discriminative stimulus effects and the magnitude of this effect was related to the number of pretreatment injections administered. Two pretreatment injections of the training dose of nicotine (0.032 mg/kg, iv) produced a 5.4-fold rightward shift in the nicotine dose-response function and a sevenfold rightward shift in the varenicline dose-response function. The duration of tolerance under these conditions was less than 60 min. These results demonstrate that tolerance to the discriminative stimulus effects of nicotine can be produced by acute nicotine exposure. Acute cross-tolerance from nicotine to varenicline is consistent with similar actions at nAChRs, and suggests that conditions resulting in acute nicotine tolerance could impact sensitivity to other nAChR agonists.” (Moerke and McMahon 2018).

According to Directive 2014/40/EU, nicotine is a highly addictive substance.

“The addictiveness of nicotine depends on the delivery system.”

“As e-cigarettes have evolved, their nicotine delivery has improved. This could mean that their addiction potential has increased, but this may also make them more attractive to smokers as a replacement for smoking. It is not yet clear how addictive e-cigarettes are, or could be, relative to tobacco cigarettes.”

“Overall, the addictiveness of NRT is much lower than that of cigarettes, with only a very small proportion of those who use these products persevering with use for a year or longer. Around 10% of nasal nicotine spray users will use for a year or longer, 5% of those using oral nicotine products, and fewer for the patch (68).”

“Consistent with the above, the RCP report concluded that there was no substantial evidence of non-smokers becoming dependent on NRT.”

“At low doses, nicotine is a stimulant. However, tolerance develops quickly and chronic exposure results in neuroadaptations, causing withdrawal effects. Addictiveness may be related to the severity of these negative withdrawal symptoms. Nicotine has complex effects, caused by its binding to and desensitizing nicotine acetylcholine receptors, and facilitating the release of a variety of neurotransmitters, including dopamine. Dopamine acts as a positive reinforcer, is involved in other addictive drug use, and is likely to underpin the pleasure that smokers report from smoking. Addictiveness and pleasure are likely to be intertwined. Pleasure is rarely reported from NRT users, but has been reported by EC users (87). It can be hard, however, to distinguish positive reward and relief from incipient withdrawal.”

“In summary, nicotine addictiveness depends on a number of factors including presence of other chemicals, speed of delivery, pH, rate of absorption, the dose, and other aspects of the nicotine delivery system, environment and behaviour. Tobacco smoking with rapid delivery of nicotine to the lungs and absorption, has been demonstrated to be highly addictive, compared with the NRT patch, for example, which has much lower dependence potential and long term use. Addictiveness is related to pleasure as well as severity of withdrawal discomfort, which are hard to tease apart. The addictive potential of other nicotine products is likely to be within the two extremes set by the cigarette and NRT patch, with some products, eg snus, also being addictive. It is thus inaccurate to say that nicotine per se is highly addictive, such statements need to be more nuanced, as addictiveness is dependent on the delivery system.” (Public Health England, 2018)

“BACKGROUND: Nicotine addiction supports tobacco smoking, a main preventable cause of disease and death in Western countries. It develops through long-term neuroadaptations in the brain reward circuit by modulating intracellular pathways and regulating gene expression. This study assesses the regional expression of the transcripts of the CRF transmission in a nicotine sensitization model, since it is hypothesised that the molecular neuroadaptations that mediate the development of sensitization contribute to the development of addiction. METHODS: Rats received intraperitoneal nicotine administrations (0.4 mg/kg) once daily for either 1 day or over 5 days. Locomotor activity was assessed to evaluate the development of sensitization. The mRNA expression of CRF and CRF1 and CRF2 receptors was measured by qPCR in the ventral mesencephalon, ventral striatum, dorsal striatum (DS), prefrontal cortex (PFCx), and hippocampus (Hip). RESULTS: Acute nicotine administration increased locomotor activity in rats. In the sub-chronic group, locomotor activity progressively increased and reached a clear sensitization. Significant effects of sensitization on CRF mRNA levels were detected in the DS (increasing effect). Significantly higher CRF1 and CRF2 receptor levels after sensitization were detected in the Hip. Additionally, CRF2 receptor levels were augmented by sensitization in the PFCx, and treatment and time-induced increases were detected in the DS. Nicotine treatment effects were observed on CRF1R levels in the DS. CONCLUSIONS: This study suggests that the CRF transmission, in addition to its role in increasing withdrawal-related anxiety, may be involved in the development of nicotine-habituated behaviours through reduced control of impulses and the aberrant memory plasticity characterising addiction.” (Carboni et al. 2018).

“Introduction: Smoking is associated with significant morbidity and mortality. Understanding the neurobiology of the rewarding effects of nicotine promises to aid treatment development for nicotine dependence. Through its actions on mesolimbic dopaminergic systems, nicotine engenders enhanced responses to drug-related cues signaling rewards, a mechanism hypothesized to underlie the development and maintenance of nicotine addiction. Methods: We evaluated the effects of acute nicotine on neural responses to anticipatory cues signaling (nondrug) monetary reward or loss among 11 nonsmokers who had no prior history of tobacco smoking. In a double-blind, crossover design, participants completed study procedures while wearing nicotine or placebo patches at least 1 week apart. In each drug condition, participants underwent functional magnetic resonance imaging while performing the monetary incentive delay task and performed a probabilistic monetary reward task, probing reward responsiveness as measured by response bias toward a more frequently rewarded stimulus. Results: Nicotine administration was associated with enhanced activation, compared with placebo, of right fronto-anterior insular cortex and striatal regions in response to cues predicting possible rewards or losses and to dorsal anterior cingulate for rewards. Response bias toward rewarded stimuli correlated positively with insular activation to anticipatory cues. Conclusion: Nicotinic enhancement of monetary reward-related brain activation in the insula and striatum in nonsmokers dissociated acute effects of nicotine from effects on reward processing due to chronic smoking. Reward responsiveness predicted a greater nicotinic effect on insular activation to salient stimuli. Implications: Previous research demonstrates that nicotine enhances anticipatory responses to rewards in regions targeted by midbrain dopaminergic systems. The current study provides evidence that nicotine also enhances responses to rewards and losses in the anterior insula. A previous study found enhanced insular activation to rewards and losses in smokers and ex-smokers, a finding that could be due to nicotine sensitization or factors related to current or past smoking. Our finding of enhanced anterior insula response after acute administration of nicotine in nonsmokers provides support for nicotine-induced sensitization of insular response to rewards and losses.” (Moran et al. 2018).

“BACKGROUND: Animal models are needed to inform FDA regulation of electronic cigarettes (ECs) because they avoid limitations associated with human studies. We previously reported that an EC refill liquid produced less aversive/anhedonic effects at a high nicotine dose than nicotine alone as measured by elevations in intracranial self-stimulation (ICSS) thresholds, which may reflect the presence of behaviorally active non-nicotine constituents (e.g., propylene glycol) in the EC liquids. The primary objective of this study was to assess the generality of our prior ICSS findings to two additional EC liquids. We also compared effects of "nicotine-free" varieties of these EC liquids on ICSS, as well as binding affinity and/or functional activity of nicotine alone, nicotine-containing EC liquids, and "nicotine-free" EC liquids at nicotinic acetylcholine receptors (nAChRs). METHODS AND RESULTS: Nicotine alone and nicotine dose-equivalent concentrations of both nicotine-containing EC liquids produced similar lowering of ICSS thresholds at low to moderate nicotine doses, indicating similar reinforcement-enhancing effects. At high nicotine doses, nicotine alone elevated ICSS thresholds (a measure of anhedonia-like behavior) while the EC liquids did not. Nicotine-containing EC liquids did not differ from nicotine alone in terms of binding affinity or functional activity at nAChRs. "Nicotine-free" EC liquids did not affect ICSS, but bound with low affinity at some (e.g., α4ß2) nAChRs. CONCLUSIONS: These findings suggest that non-nicotine constituents in these EC liquids do not contribute to their reinforcement-enhancing effects. However, they may attenuate nicotine's acute aversive/anhedonic and/or toxic effects, which may moderate the abuse liability and/or toxicity of ECs.” (Harris et al. 2018).

“RATIONALE: Prolonged use of nicotine appears to enhance incentive salience, a motivational-cognitive process that transforms an otherwise neutral stimulus into a "wanted" stimulus. It has been suggested that nicotinic enhancement of incentive salience contributes to the potential of relapse in individuals with tobacco addiction. However, there are two main limitations of prior research that caution this claim: (a) the use of passive experimentally delivered nicotine and (b) the use of sign-tracking as an index of incentive salience, without acknowledging the competing nature of goal- and sign-tracking responses. OBJECTIVES: To determine whether nicotinic enhancement of incentive salience attributed to non-nicotinic stimuli occurs when rats self-administer nicotine, and whether it is facilitated by a prior history of nicotine self-administration. METHODS: Twenty-three male rats were trained daily, for 24 days, on a nicotine self-administration (SA) paradigm in the morning, and on a four-conditioned-stimuli Pavlovian conditioned approach (4-CS PCA) task in the afternoon. Self-administration was followed by extinction and cue reinstatement. A subcutaneous nicotine challenge was performed during the last 7 days of the study. RESULTS: Nicotine self-administration selectively enhanced sign-tracking in the 4-CS PCA. Upon extinction, sign-tracking quickly declined to control levels. Experimenter-administered nicotine enhanced sign-tracking similarly regardless of nicotine history. CONCLUSIONS: The results suggest that nicotinic enhancement of incentive salience is transient, and a previous history of nicotine use does not cause further sensitization. Taken together, these results suggest that nicotine enhances incentive salience, particularly-and perhaps exclusively-while onboard.” (Overby et al. 2018).

“RATIONALE AND OBJECTIVES: A potential reason that cigarette smoking can persist despite multiple quit attempts is that repeated voluntary nicotine intake may facilitate a transition from goal-directed to habitual behavioral control. Although accelerated habit formation for self-administered ethanol or cocaine has been previously demonstrated, this phenomenon has not been extensively studied with nicotine. We therefore examined the liability of nicotine self-administration to become habitual, while also examining that of orally consumed saccharin as an experimental control. METHODS: Under fixed ratio 1 (FR-1) schedules, male Sprague-Dawley rats (n = 8-11/group) lever-pressed for intravenous (IV) nicotine (30 μg/kg/infusion) for 10 consecutive days, while also lever-pressing for saccharin solution (0.1% w/v, 0.19 mL/delivery) in separate operant sessions. In experiment 1, either nicotine or saccharin was devalued by pairing with the aversive agent lithium chloride (LiCl; 0.15 M, 14.1 mL/kg) prior to extinction and reacquisition testing. In experiment 2, the contingency between lever pressing and delivery of either nicotine or saccharin was degraded in six sessions, followed by extinction testing. RESULTS: LiCl pairings selectively reduced responding for nicotine (-35% from control) and saccharin (-48%) in reacquisition testing, indicating that both rewards were effectively devalued. During extinction testing, saccharin-seeking responses were reduced by both manipulations (devaluation -30%, degradation -79%), suggesting that responding for saccharin was goal-directed. In contrast, nicotine-seeking responses were not significantly affected by either manipulation (devaluation -4%, degradation -21%), suggesting that responding for nicotine was habitually driven. CONCLUSIONS: Operant responding for IV nicotine may rapidly come under habitual control, potentially contributing to the tenacity of tobacco use.” (Loughlin et al. 2017).

“Chronic tobacco use dramatically increases health burdens and financial costs. Limitations of current smoking cessation therapies indicate the need for improved molecular targets. The main addictive component of tobacco, nicotine, exerts its dependency effects via nicotinic acetylcholine receptors (nAChRs). Activation of the homomeric α7 nAChR reduces nicotine's rewarding properties in conditioned place preference (CPP) test and i.v. self-administration models, but the mechanism underlying these effects is unknown. Recently, the nuclear receptor peroxisome proliferator-activated receptor type-α (PPARα) has been implicated as a downstream signaling target of the α7 nAChR in ventral tegmental area dopamine cells. The present study investigated PPARα as a possible mediator of the effect of α7 nAChR activation in nicotine dependence. Our results demonstrate the PPARα antagonist GW6471 blocks actions of the α7 nAChR agonist PNU282987 on nicotine reward in an unbiased CPP test in male ICR adult mice. These findings suggests that α7 nAChR activation attenuates nicotine CPP in a PPARα-dependent manner. To evaluate PPARα activation in nicotine dependence we used the selective and potent PPARα agonist, WY-14643 and the clinically used PPARα activator, fenofibrate, in nicotine CPP and we observed attenuation of nicotine preference, but fenofibrate was less potent. We also studied PPARα in nicotine dependence by evaluating its activation in nicotine withdrawal. WY-14643 reversed nicotine withdrawal signs whereas fenofibrate had modest efficacy. This suggests that PPARα plays a role in nicotine reward and withdrawal and that further studies are warranted to elucidate its function in mediating the effects of α7 nAChRs in nicotine dependence.” (Jackson et al. 2017).

“Delta and kappa opioid receptors (DOR and KOR, respectively) and their endogenous ligands, proenkephalin (PENK) and prodynorphin (PDYN)-derived opioid peptides are proposed as important mediators of nicotine reward. This study investigated the regulatory effect of chronic nicotine treatment on the gene expression of DOR, KOR, PENK and PDYN in the mesocorticolimbic system. Three groups of rats were injected subcutaneously with nicotine at doses of 0.2, 0.4, or 0.6 mg/kg/day for 6 days. Rats were decapitated 1 hr after the last dose on day six, as this timing coincides with increased dopamine release in the mesocorticolimbic system. mRNA levels in the ventral tegmental area (VTA), lateral hypothalamic area (LHA), amygdala (AMG), dorsal striatum (DST), nucleus accumbens, and medial prefrontal cortex were measured by quantitative real-time PCR. Our results showed that nicotine upregulated DOR mRNA in the VTA at all of the doses employed, in the AMG at the 0.4 and 0.6 mg/kg doses, and in the DST at the 0.4 mg/kg dose. Conversely, PDYN mRNA was reduced in the LHA with 0.6 mg/kg nicotine and in the AMG with 0.4 mg/kg nicotine. KOR mRNA was also decreased in the DST with 0.6 mg/kg nicotine. Nicotine did not regulate PENK mRNA in any brain region studied.” (Ugur et al. 2017).

# *8.* *Burnt ingredient toxicity*

Not required.

“Tobacco heating products (THPs) represent a subset of the next-generation nicotine and tobacco product category, in which tobacco is typically heated at temperatures of 250-350 °C, thereby avoiding many of the harmful combustion-related toxicant emissions of conventional cigarettes. In this study, we have assessed aerosol generation and cytotoxicity from two commercially available THPs, THP1.0 and THS, relative to tobacco smoke from 3R4F reference cigarettes, using an adapted Borgwaldt RM20S Smoking Machine. Quantification of nicotine in the exposed cell-culture media showed greater delivery of nicotine from both THPs than from the cigarette. Using Neutral Red Uptake assay, THPs demonstrated reduced in vitro cytotoxicity in H292 human bronchial epithelial cells as compared with 3R4F cigarette exposure at the air-liquid interface (p < 0.0001). Both THPs demonstrated a statistically similar reduction in biological response, with >87% viability relative to 3R4F at a common aerosol dilution (1:40, aerosol:air). A similar response was observed when plotted against nicotine; a statistical difference between 3R4F and THPs (p < 0.0001) and no difference between the THPs (p = 0.0186). This pre-clinical in vitro biological testing forms part of a larger package of data to help assess the safety and risk reduction potential of next-generation tobacco products relative to cigarettes, using a weight of evidence approach.” (Jaunky et al. 2018).

“This series of nine papers described the operation and pre-clinical assessment of a tobacco heating product THP1.0. This last paper contextualises the pre-clinical assessment data on THP1.0 with data from other next generation products relative to cigarette smoke. The tobacco and nicotine risk continuum is a concept that ranks products according to their potential harm, with cigarettes at the highest risk extreme and Nicotine Replacement Therapy at the least risky extreme. Data generated in pre-clinical studies on THP1.0 and a range of Next Generation Products (NGPs) may provide some initial indication of potential ranking of these products, although importantly, data from such studies are limited and cannot take into consideration several important aspects for risk such as long term product use patterns. In each of the studies, the responses to the emissions from THP1.0 were substantially reduced relative to cigarette smoke. Additionally, responses from THP1.0 were very similar to those from the other NGP emissions. A comparison of the results clearly showed the emissions from all the NGPs were considerably lower than those from cigarettes and all in around the same emissions level. These results show that THP1.0 could have the potential to be a reduced risk product compared to cigarettes, though further studies assessing the exposure, individual and population risk reduction profile would be required to substantiate this potential.” (Murphy et al. 2018).

“The use of novel tobacco- and nicotine-containing vapor products that do not combust tobacco leaves is on the rise worldwide. The emissions of these products typically contain lower numbers and levels of potentially harmful chemicals compared with conventional cigarette smoke. These vapor products may therefore elicit fewer adverse biological effects. We compared the effects of emissions from different types of such products, i.e., our proprietary novel tobacco vapor product (NTV), a commercially available heat-not-burn tobacco product (HnB), and e-cigarette (E-CIG), and a combustible cigarette in a human bronchial epithelial cell line. The aqueous extract (AqE) of the test product was prepared by bubbling the produced aerosol into medium. Cells were exposed to the AqEs of test products, and then glutathione oxidation, Nrf2 activation, and secretion of IL-8 and GM-CSF were examined. We found that all endpoints were similarly perturbed by exposure to each AqE, but the effective dose ranges were different between cigarette smoke and the tobacco- and nicotine-containing vapors. These results demonstrate that the employed assays detect differences between product exposures, and thus may be useful to understand the relative potential biological effects of tobacco- and nicotine-containing products.” (Munakata et al. 2018).

“Electronic cigarette (e-cigarette) usage in the USA has drastically increased in the past 5 years due to age restrictions on conventional cigarettes, aggressive marketing and a perception that e-cigarettes are a healthy alternative. E-cigarettes contain nicotine, water, glycerol, propylene glycol and optional flavouring. On inhalation, the device heats the ingredients into a vapour. While tobacco cigarette smoke is known to cause deleterious effects on the cardiovascular system, angiogenesis and skin capillary perfusion by causing direct injury to blood vessel walls, increased platelet aggregation, microvascular thrombosis and inflammation, the consequences of e-cigarette vapour exposure on the lung are still largely unexplored. Recently, Lerner et al 2015. reported that vapours produced by e-cigarettes and e-cigarette fluids with flavourings induced toxicity, oxidative stress and inflammatory response in human bronchial airway epithelial cells (H292) and fetal lung fibroblasts (HFL1) as well as mouse lung. Garcia-Arcos et al. 2016 showed that the aerosolised nicotine-containing e-cigarette fluid increased airway hyperreactivity, distal airspace enlargement, mucin production, and cytokine and protease expression in mice, implying potential dangers of nicotine inhalation during e-cigarette use. The inflammatory response to e-cigarette use involved increased neutrophil activation and mucus production, and decreased mucociliary clearance. In human embryonic and mouse neural stem cells, human pulmonary fibroblasts, and skin and lung cells, cytotoxicity of e-cigarette vapour was correlated with the number and concentration of chemicals used to flavour the fluids. We recently showed in the skin flap survival model in vivo that nicotine-containing e-cigarette vapour is just as harmful to the microcirculation as tobacco cigarette smoke.” (Reinikovaite eta l. 2018).

“Nicotine is the major neurotoxicant in cigarettes that affects many transmitter systems within the brain as well as other factors, including the growth factors. Brain derived neurotrophic factor (BDNF), is the most abundant growth factor in the brain and plays a critical role in early new neuron differentiation, development and synapsis growth, and the survival of fully developed neurons and synaptic activity. Over the past 3 decades, data has emerged on the effects of nicotine and cigarette smoke exposure on the expression of BDNF and its primary specific receptor tyrosine kinase receptor B (TrkB). This review summarizes data regarding the changes in brain BDNF expression after nicotine or cigarette smoke exposure, and discusses their implications considering BDNF's functional roles.” (Machaalani and Chen 2018).

“Recent advancements in in vitro exposure systems and cell culture technology enable direct exposure to cigarette smoke (CS) of human organotypic bronchial epithelial cultures. MucilAir organotypic bronchial epithelial cultures were exposed, using a Vitrocell exposure system, to mainstream aerosols from the 3R4F cigarette or from a recently developed novel tobacco vapor product (NTV). The exposure aerosol dose was controlled by dilution flow and the number of products smoked; there were five exposure conditions for 3R4F smoke and three for NTV vapor. The amount of nicotine delivered to the tissues under each condition was analyzed and that of the total particulate matter (TPM) was estimated using nicotine data. The nicotine dose was similar for the two products at the highest dose, but the estimated TPM levels from the NTV were 3.7 times the levels from the 3R4F. Following 3R4F smoke exposure, a dose dependent increase was observed in cytotoxicity, cytokine secretion, and differential gene expression. However, no changes were detected in these endpoints following NTV vapor exposure, suggesting the biological effects of NTV vapor are lower than those of conventional combustible CS. Our study design, which includes collection of biological data and dosimetry data, is applicable to assessing novel tobacco products.” (Ishikawa et al. 2018).

“Long-term exposure to cigarette smoke induces severe injuries to respiratory system through several mechanisms, some of them are well defined, but many others are not yet elucidated. Beside its classical role in nervous system, we have previously shown that Nerve Growth Factor (NGF) and its receptors have a crucial role in airway inflammatory diseases, such as Chronic Obstructive Pulmonary Disease. To expand our knowledge about the relevance of NGF and its receptors in airway diseases induced by cigarette smoking, we exposed for 16 weeks the bronchial epithelial cell line BEAS-2B to sub-toxic concentrations of whole cigarette smoke extract or pure nicotine. Viability, cell cycle gene expression, cell morphology and migration ability were tested and compared to NGF release and gene expression. Modulation of its receptors TrKA and p75NTR was also analyzed. The present study shows that long term exposure of BEAS-2B cells to cigarette smoke extract or nicotine induces: (A) differences: in cell viability, in the expression of cell cycle-related genes, in NGF release and in gene expression of NGF and its receptors; (B) similarities: in morphology and migration ability. Taken together, our data provide new insights about the biological role of NGF and its receptors in airway diseases induced by long-term cigarette smoking and, finally, our data evidence the opportunity not to use nicotine lozenges or e-cigarettes as anti smoking replacement therapy in patients with a previous airway disease according to the ability of nicotine to increase the amount of the pro-inflammatory cytokine NGF into the bronchial environment.” (Stabile et al. 2018).

“The introduction of electronic cigarettes has led to widespread discussion on the cardiovascular risks compared to conventional smoking. We therefore conducted a randomized cross-over study of the acute use of three tobacco products, including a control group using a nicotine-free liquid. Fifteen active smokers were studied during and after smoking either a cigarette or an electronic cigarette with or without nicotine (eGo-T CE4 vaporizer). Subjects were blinded to the nicotine content of the electronic cigarette and were followed up for 2 hours after smoking a cigarette or vaping an electronic cigarette. Peripheral and central blood pressures as well as parameters of arterial stiffness were measured by a Mobil-O-Graph® device. The peripheral systolic blood pressure rose significantly for approximately 45 minutes after vaping nicotine-containing liquid (p<0.05) and for approximately 15 minutes after smoking a conventional cigarette (p<0.01), whereas nicotine-free liquids did not lead to significant changes during the first hour of follow-up. Likewise, heart rate remained elevated approximately 45 minutes after vaping an electronic cigarette with nicotine-containing liquid and over the first 30 minutes after smoking a cigarette in contrast to controls. Elevation of pulse wave velocity was independent from mean arterial pressure as well as heart rate in the electronic cigarette and cigarette groups. In this first of its kind trial, we observed changes in peripheral and central blood pressure and also in pulse wave velocity after smoking a cigarette as well as after vaping a nicotine-containing electronic cigarette. These findings may be associated with an increased long-term cardiovascular risk.” (Franzen et al. 2018).

# *9.* *Heated/vapor emissions toxicity*

“Introduction: Novel nicotine delivery systems represent an evolving part of the tobacco harm reduction strategy. The pharmacokinetic (PK) profile of nicotine delivered by P3L, a pulmonary nicotine delivery system, and its effects on smoking urges and craving relief in relation to Nicorette inhalator were evaluated. Methods: This open-label, ascending nicotine levels study was conducted in 16 healthy smokers. Three different nicotine delivery levels, 50, 80, and 150 µg/puff, delivered by the P3L system were evaluated consecutively on different days after the use of the Nicorette inhalator. Venous nicotine PK, subjective effects, and tolerability were assessed. Results: Geometric least-squares means for maximum plasma nicotine concentration (Cmax), generated by the mixed-effect model for exposure comparison, were 9.7, 11.2, and 9.8 ng/mL for the 50, 80, and 150 µg/puff P3L variants, respectively, compared to 6.1 ng/mL after Nicorette inhalator use. Median time from product use start to Cmax was 7.0 minutes for all P3L, compared to 30.0 minutes for the Nicorette inhalator. Craving reduction was slightly faster than with the Nicorette inhalator as assessed with the visual analog scale craving score. The mean Questionnaire of Smoking Urges -brief total scores did not differ for both products. P3L was well tolerated. Conclusions: At all three nicotine levels tested, the inhalation of the nicotine lactate aerosol delivered with the P3L provided plasma nicotine concentrations higher and faster compared to the Nicorette inhalator. The plasma nicotine concentration-time profile supports a pulmonary route of absorption for P3L compared to the oromucosal absorption of the Nicorette inhalator. Implications: The combination of nicotine and lactic acid with the P3L device shows potential over existing nicotine delivery systems by delivering nicotine with kinetics close to published data on conventional cigarettes and without exogenous carrier substances as used in current electronic nicotine delivery systems. Altogether, the PK profile, subjective effects, and safety profile obtained in this study suggest P3L is an innovative nicotine delivery product that will be acceptable to adult smokers as an alternative to cigarettes.” (Teichert et al. 2018).

“E-cigarette usage is increasing, especially among the young, with both the general population and physicians perceiving them as a safe alternative to tobacco smoking. Worryingly, e-cigarettes are commonly used by pregnant women. As nicotine is known to adversely affect children in utero, we hypothesized that nicotine delivered via e-cigarettes would negatively affect lung development. To test this, we developed a mouse model of maternal e-vapor (nicotine and nicotine-free) exposure and investigated the impact on the growth and lung inflammation in both offspring and mothers. Female Balb/c mice were exposed to e-fluid vapor containing nicotine (18 mg/ml nicotine E-cigarette [E-cig18], equivalent to two cigarettes per treatment, twice daily,) or nicotine free (E-cig0 mg/ml) from 6 weeks before mating until pups weaned. Male offspring were studied at Postnatal Day (P) 1, P20, and at 13 weeks. The mothers were studied when the pups weaned. In the mothers' lungs, e-cigarette exposure with and without nicotine increased the proinflammatory cytokines IL-1β, IL-6, and TNF-α. In adult offspring, TNF-α protein levels were increased in both E-cig18 and E-cig0 groups, whereas IL-1β was suppressed. This was accompanied by global changes in DNA methylation. In this study, we found that e-cigarette exposure during pregnancy adversely affected maternal and offspring lung health. As this occurred with both nicotine-free and nicotine-containing e-vapor, the effects are likely due to by-products of vaporization rather than nicotine.” (Chen H et al. 2018).

“Tobacco heating products (THPs) represent a subset of the next-generation nicotine and tobacco product category, in which tobacco is typically heated at temperatures of 250-350 °C, thereby avoiding many of the harmful combustion-related toxicant emissions of conventional cigarettes. In this study, we have assessed aerosol generation and cytotoxicity from two commercially available THPs, THP1.0 and THS, relative to tobacco smoke from 3R4F reference cigarettes, using an adapted Borgwaldt RM20S Smoking Machine. Quantification of nicotine in the exposed cell-culture media showed greater delivery of nicotine from both THPs than from the cigarette. Using Neutral Red Uptake assay, THPs demonstrated reduced in vitro cytotoxicity in H292 human bronchial epithelial cells as compared with 3R4F cigarette exposure at the air-liquid interface (p < 0.0001). Both THPs demonstrated a statistically similar reduction in biological response, with >87% viability relative to 3R4F at a common aerosol dilution (1:40, aerosol:air). A similar response was observed when plotted against nicotine; a statistical difference between 3R4F and THPs (p < 0.0001) and no difference between the THPs (p = 0.0186). This pre-clinical in vitro biological testing forms part of a larger package of data to help assess the safety and risk reduction potential of next-generation tobacco products relative to cigarettes, using a weight of evidence approach.” (Jaunky et al. 2018).

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“Recent advancements in in vitro exposure systems and cell culture technology enable direct exposure to cigarette smoke (CS) of human organotypic bronchial epithelial cultures. MucilAir organotypic bronchial epithelial cultures were exposed, using a Vitrocell exposure system, to mainstream aerosols from the 3R4F cigarette or from a recently developed novel tobacco vapor product (NTV). The exposure aerosol dose was controlled by dilution flow and the number of products smoked; there were five exposure conditions for 3R4F smoke and three for NTV vapor. The amount of nicotine delivered to the tissues under each condition was analyzed and that of the total particulate matter (TPM) was estimated using nicotine data. The nicotine dose was similar for the two products at the highest dose, but the estimated TPM levels from the NTV were 3.7 times the levels from the 3R4F. Following 3R4F smoke exposure, a dose dependent increase was observed in cytotoxicity, cytokine secretion, and differential gene expression. However, no changes were detected in these endpoints following NTV vapor exposure, suggesting the biological effects of NTV vapor are lower than those of conventional combustible CS. Our study design, which includes collection of biological data and dosimetry data, is applicable to assessing novel tobacco products.” (Ishikawa et al. 2018).

“The introduction of electronic cigarettes has led to widespread discussion on the cardiovascular risks compared to conventional smoking. We therefore conducted a randomized cross-over study of the acute use of three tobacco products, including a control group using a nicotine-free liquid. Fifteen active smokers were studied during and after smoking either a cigarette or an electronic cigarette with or without nicotine (eGo-T CE4 vaporizer). Subjects were blinded to the nicotine content of the electronic cigarette and were followed up for 2 hours after smoking a cigarette or vaping an electronic cigarette. Peripheral and central blood pressures as well as parameters of arterial stiffness were measured by a Mobil-O-Graph® device. The peripheral systolic blood pressure rose significantly for approximately 45 minutes after vaping nicotine-containing liquid (p<0.05) and for approximately 15 minutes after smoking a conventional cigarette (p<0.01), whereas nicotine-free liquids did not lead to significant changes during the first hour of follow-up. Likewise, heart rate remained elevated approximately 45 minutes after vaping an electronic cigarette with nicotine-containing liquid and over the first 30 minutes after smoking a cigarette in contrast to controls. Elevation of pulse wave velocity was independent from mean arterial pressure as well as heart rate in the electronic cigarette and cigarette groups. In this first of its kind trial, we observed changes in peripheral and central blood pressure and also in pulse wave velocity after smoking a cigarette as well as after vaping a nicotine-containing electronic cigarette. These findings may be associated with an increased long-term cardiovascular risk.” (Franzen et al. 2018).

“E-cigarettes are battery-powered electronic devices from which users can inhale nicotine following its aerosolisation from a liquid solution. Some regulators and public health bodies consider e-cigarettes as potentially playing a major role in tobacco harm reduction. Their ability to provide nicotine to smokers in both amount and in a manner and form generally similar to cigarette smoking have been proposed as key components to help smokers reduce or cease the use of combustible cigarettes. Nicotine pharmacokinetic studies of e-cigarettes have been performed for a number of years and are beginning to show how nicotine delivery is evolving as the products themselves evolve. In this review, we provide a critical overview of the literature to describe what is known about nicotine delivery from e-cigarettes. We will discuss how the progression of e-cigarette design, development, and user familiarity has allowed increases in nicotine availability to the user, in the context of how much and how rapidly nicotine is delivered during acute-use periods. This review will also provide insight into current research gaps and highlight the potential utility of modelling and the standardisation of methodologies used to assess nicotine delivery to facilitate identification of products that are best suited to displace cigarette smoking among adult smokers.” (Fearon et al. 2018).

“A prototype electronic cigaret device and three formulations were evaluated in a 90-day rat inhalation study followed by a 42-day recovery period. Animals were randomly assigned to groups for exposure to low-, mid- and high-dose levels of aerosols composed of vehicle (glycerin and propylene glycol mixture); vehicle and 2.0% nicotine; or vehicle, 2.0% nicotine and flavor mixture. Daily targeted aerosol total particulate matter (TPM) doses of 3.2, 9.6 and 32.0 mg/kg/day were achieved by exposure to 1 mg/L aerosol for 16, 48 and 160 min, respectively. Pre-study evaluations included indirect ophthalmoscopy, virology and bacteriological screening. Body weights, clinical observations and food consumption were monitored weekly. Plasma nicotine and cotinine and carboxyhemoglobin levels were measured at days 28 and 90. After days 28, 56 and 90, lung function measurements were obtained. Biological endpoints after 90-day exposure and 42-day recovery period included clinical pathology, urinalysis, bronchoalveolar fluid (BALF) analysis, necropsy and histopathology. Treatment-related effects following 90 days of exposure included changes in body weight, food consumption and respiratory rate. Dose-related decreases in thymus and spleen weights, and increased BALF lactate dehydrogenase, total protein, alveolar macrophages, neutrophils and lung weights were observed. Histopathology evaluations revealed sporadic increases in nasal section 1-4 epithelial hyperplasia and vacuolization. Following the recovery period, effects in the nose and BALF were persistent while other effects were resolved. The no observed effect level based upon body weight decreases is considered to be the mid-dose level for each formulation, equivalent to a daily TPM exposure dose of approximately 9.6 mg/kg/day.” (Werley et al. 2016).

“A new review of possible effects of nicotine in EC on cardiovascular function concluded that short-term use of EC appeared to pose low cardiovascular risk in healthy users (72). The authors commented that some adverse effects may exist in people with preexisting CVD, though these would be lower than risks of smoking. The concern is based on a finding that although snus use does not increase CVD risks, among people who suffer a myocardial infarction, those who continue using snus have lower survival rates compared to those who quit snus. This could be due to post-myocardial infarction nicotine use. However, it is also possible that people unable to stop tobacco use despite suffering a myocardial infarction are typically highly dependent and this is associated with lower socioeconomic status, less access to health care and a possibility that they seek help later than non-tobacco users, have higher levels of stress, and a range of lifestyle behaviours detrimental to health. Studies controlling for such factors are needed to clarify this issue.” (Public Health England, 2018)

“Smokeless tobacco (SLT) products are consumed by millions of people in over 130 countries around the world. Consumption of SLT has been estimated to cause a number of diseases accounting to more than 0.65 million deaths per year. There is sufficient epidemiological evidence on the association of SLT products with nicotine addiction, cancers of oral cavity and digestive systems but there is a lack of understanding of the role of toxic chemicals in these diseases. We provide the first comprehensive in-silico analysis of chemical compounds present in different SLT products used worldwide. Many of these compounds are found to have good absorption, solubility and permeability along with mutagenic and toxic properties. They are also found to target more than 350 human proteins involved in a plethora of human biological processes and pathways. Along with all the previously known diseases, the present study has identified the association of compounds of SLT products with a number of unknown diseases like neurodegenerative, immune and cardiac diseases (Left ventricular non compaction, dilated cardiomyopathy etc). These findings indicate far-reaching impact of SLT products on human health than already known which needs further validations using epidemiological, in-vitro and in-vivo methodologies. Thus, this study will provide one stop information for the policy makers in development of regulatory policies on toxic contents of SLT products.” (Bhartiya et al 2018).

“OBJECTIVE: This paper primarily aimed to review articles which specifically quantified the risk of electronic cigarette's (e-cigarette) usage via the health risk assessment (HRA) approach. METHODS: Systematic literature searches were conducted using PubMed search engine databases. Search terms such as "electronic cigarette", "e-cigarette", "electronic nicotine delivery systems", "electronic cigarette liquid", "electronic cigarette vapors", and "health risk assessment" were used to identify the relevant articles to be included in this review. To enable comparison, hazard quotient (HQ) and lifetime cancer risk (LCR) for the chemicals measured in the selected articles were calculated for three of the articles using the formula: [1] HQ=average daily dose (ADD)/reference dose (RfD) or exposure air concentration (EC)/reference concentration (RfC); [2] LCR=lifetime average daily dose (LADD) × cancer slope factor (CSF) or exposure air concentration (EC) × inhalation unit risk (IUR). RESULTS: Four articles pertaining to HRA of e-cigarettes were critically reviewed, three of the papers focused on specific chemicals namely nicotine, propylene glycol (PG), glycerol and 1,2-propanediol, while one article evaluated the health risks posed by heavy metals contained in e-cigarettes. The calculated HQs for the chemicals in this review had large variations. HQs of the six chemicals, i.e. nicotine, PG, glycerol, cadmium, ethylene glycol, nickel, aluminum and titanium, were found to have the potential to contribute to non-carcinogenic health risks. None of the LCR calculated had risks exceeding the acceptable limit. CONCLUSION: There are limited HRA studies and the ones that were available provided inconsistent scientific evidences on the health risk characterization arising from the usage of e-cigarettes. As such, there is a need to perform more studies on HRA of e-cigarettes by using uniformed and comprehensive steps and similar reference threshold levels of exposures.” (Zulkifli et al. 2018).

# *10.* *Ecotoxicity*

## *10.1.* *Environmental fate*

According to a model of gas/particle partitioning of semi-volatile organic compounds in the atmosphere, nicotine, which has a vapor pressure of 0.038 mm Hg at 25°C, is expected to exist solely as a vapor in the ambient atmosphere.

Vapor-phase nicotine is degraded in the atmosphere by reaction with photochemically-produced hydroxyl radical and the half-life for this reaction in air is estimated to be 4 hours. Nicotine does not contain chromophores that absorb at wavelengths >290 nm and therefore is not expected to be susceptible to direct photolysis by sunlight (Bidleman T, 1988; HSDB; Meylan W & Howard P, 1993).

When released into water, nicotine is not expected to adsorb to suspended solids and sediment based upon the estimated Koc. Volatilization from water surfaces is not expected to be an important fate process based upon this compound's estimated Henry's Law constant. An estimated BCF of 3 suggests the potential for bioconcentration in aquatic organisms is low (Seckar J et al., 2008; HSDB).

## *10.2.* *Aquatic toxicity*

Nicotine was evaluated for acute aquatic toxicity in rainbow trout and daphnia. The mean 96 h LC50 for rainbow trout and the 48 h EC50 for daphnia were 4 and 0.24 mg/L, respectively. Nicotine sulfate was also evaluated in multiple aquatic species for lethality.The species and the corresponding toxicity are as follows: fathead minnow (96 h LC50=19.7 mg/L), rainbow trout (96 h LC50=7.31 mg/L), bluegill (96 h LC50=4.31 mg/L), goldfish (96 h LC50=13.1 mg/L), daphnia magna (48 h EC50=3.25 mg/L), midge (48 h LC50=427 mg/L), crayfish (96 h LC50=438.2 mg/L), and snail (96 h LC50=438.2 mg/L) (Hughes B, 2005).

In another experiment plankters were exposed to relatively low concentrations of nicotine for 168 hours and showed varied responses. The zooplankters (Cyclops, Nauplius, Daphnia and Ceriodaphnia) were killed at 2 ppm and Cypris tolerated this concentrations. Diaptomas, Gastrorica and Brachionus tolerated up to 50 ppm. All phytoplankters (Volvox, Pandorina and Closterium) survived 5 ppm (Konar SK, 1977).

Seckar J et al., (2008) determined S- (-)-nicotine-hemisulphate (S-NHS) inhibited 50% of the algal growth rate at 96 hours to be ErC50=72.9 ± 1.48 mg/L. The concentration of S-NHS inhibiting 50% of the algal biomass at 96 hours was 115 ± 1.6 mg/L and the no-observed-effect concentration was 10 mg/L.

## *10.3.* *Sediment toxicity*

No data available to us at this time.

## *10.4.* *Terrestrial toxicity*

No data available to us at this time.

## *10.5.* *All other relevant types of ecotoxicity*

No data available to us at this time.

# *11.* *References for conventional products*

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# *14.* *Last audited*

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