Animal species in drug development with emphasis on preclinical safety

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Overview

- Use of animals
- Regulations regarding species selection
- Species similarities and differences
- Commonly used species in toxicology
- Humans
- Adverse drug reactions in humans
- Extrapolation of animal data
- Species selection
- Conclusions

Overview

- Use of animals
  - History
  - Current
- Regulations regarding species selection
- Species similarities and differences
- Commonly used species in toxicology
- Humans
- Adverse drug reactions in humans
- Extrapolation of animal data
- Species selection
- Conclusions

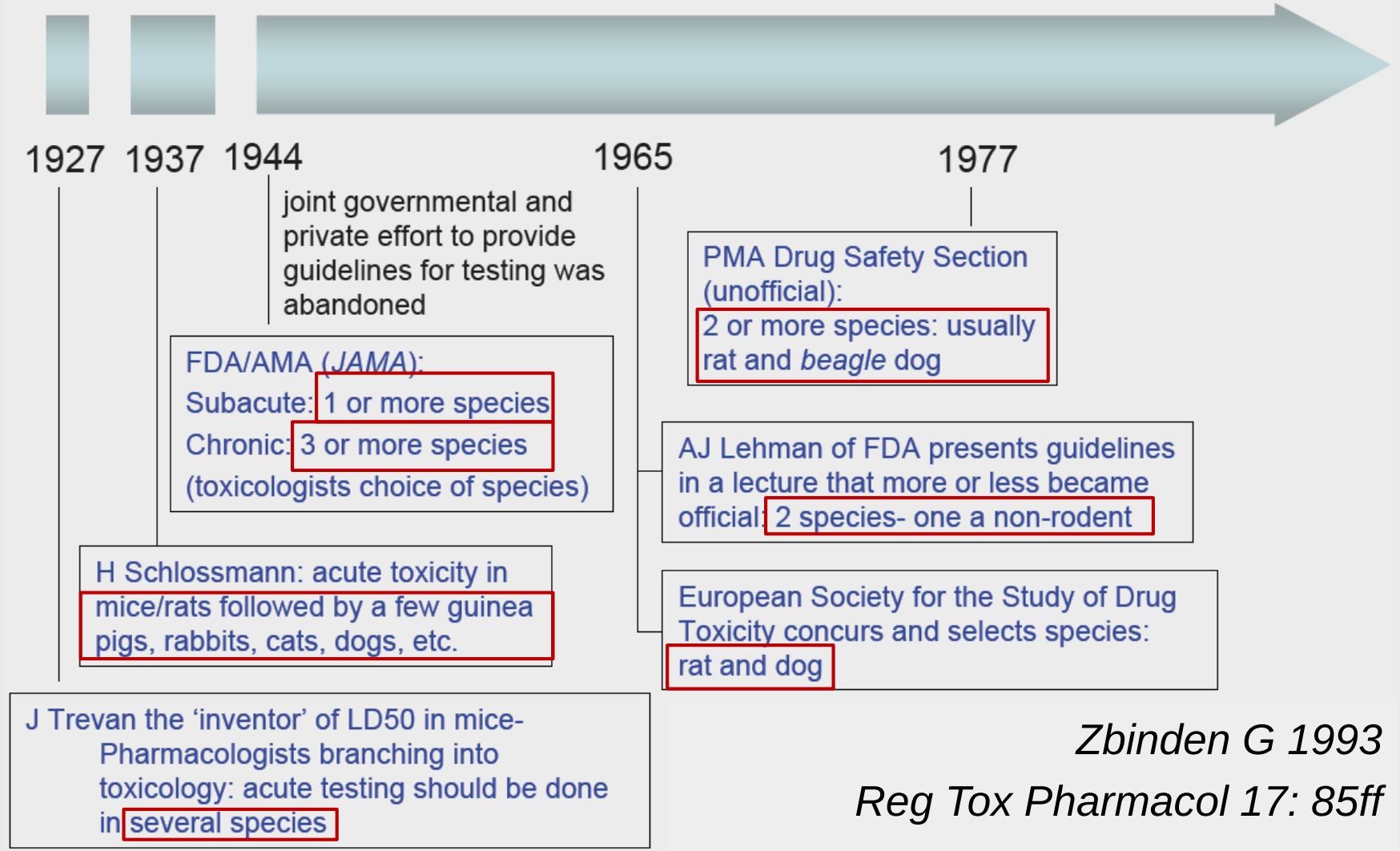
Glimpses on history of *animal use*

- Aristotle (384-322 BC) and Erasistratus (304-258 BC):
  Experiments on living animals
- Louis Pasteur in the 1880s:
  Anthrax infection of sheep for proof of germ theory
- Ivan Pavlov in the 1890s:
  Classical conditioning on dogs
- Insulin isolated from dogs in 1922
- Russian dog Laika orbited the earth in 1957

Glimpses on history of *toxicology*

- Claude Bernard (1813-1878): animal experiments are "entirely conclusive for toxicology and hygiene of man“
- 1937 - Sulfanilamide caused over 100 deaths → laws for safety testing of drugs on animals before marketing → US FDA
- 1955 - Delaney Amendment regarding carcinogenicity
- In the 1960s, thalidomide caused over 10’000 deformed children, → further safety testing on pregnant animals required

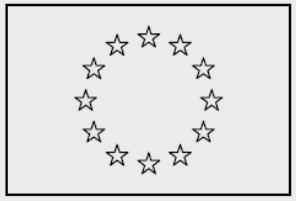
Species required over time



Zbinden G 1993

Reg Tox Pharmacol 17: 85ff

Experimental animals used in 2008



EUROPEAN COMMISSION

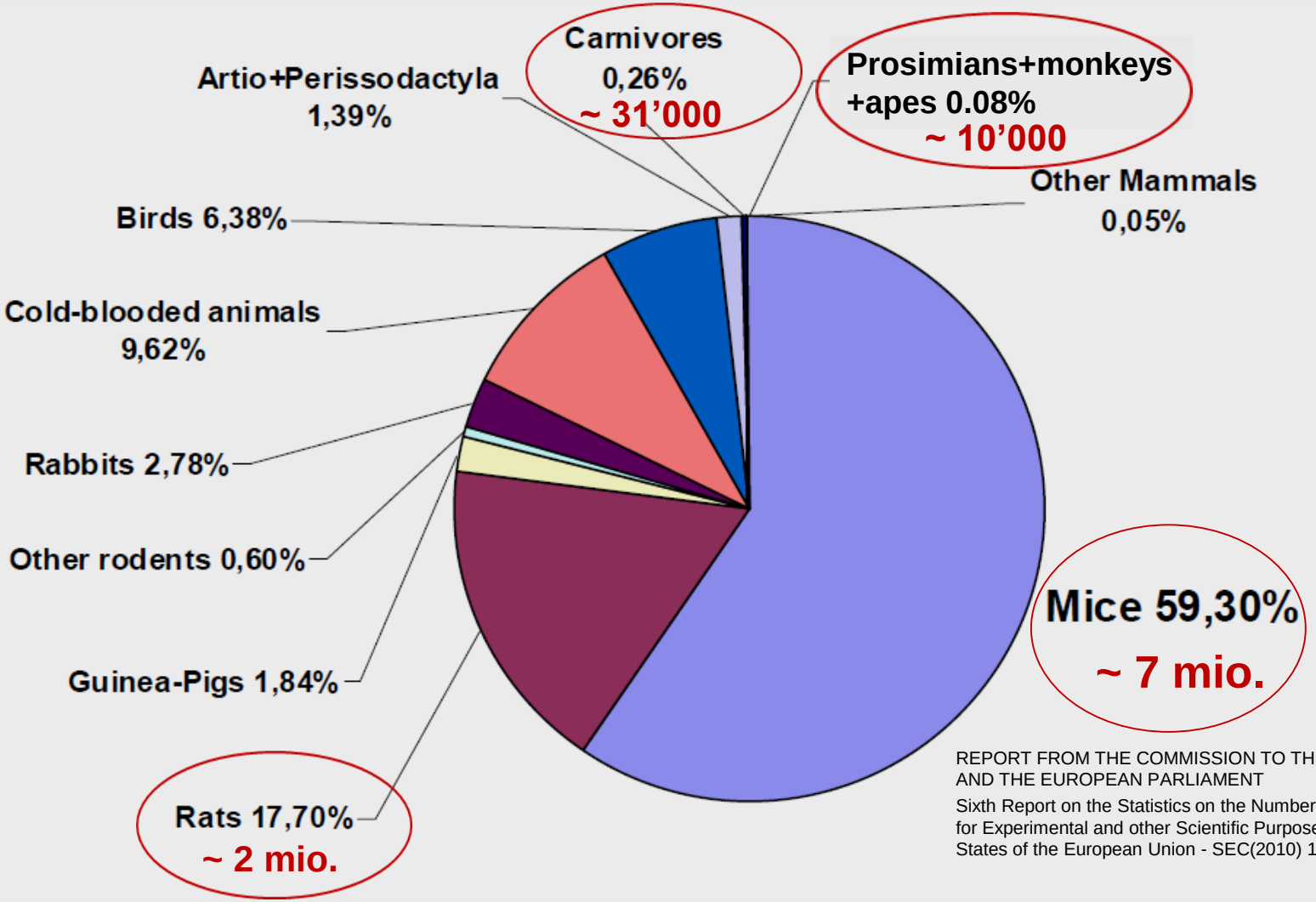
EU (27) population: ~ 500 mio.

REPORT FROM THE COMMISSION TO THE COUNCIL AND THE EUROPEAN PARLIAMENT

Sixth Report on the Statistics on the Number of Animals used for Experimental and other Scientific Purposes in the Member States of the European Union
SEC(2010) 1107

Total number of animals used: 12 millions

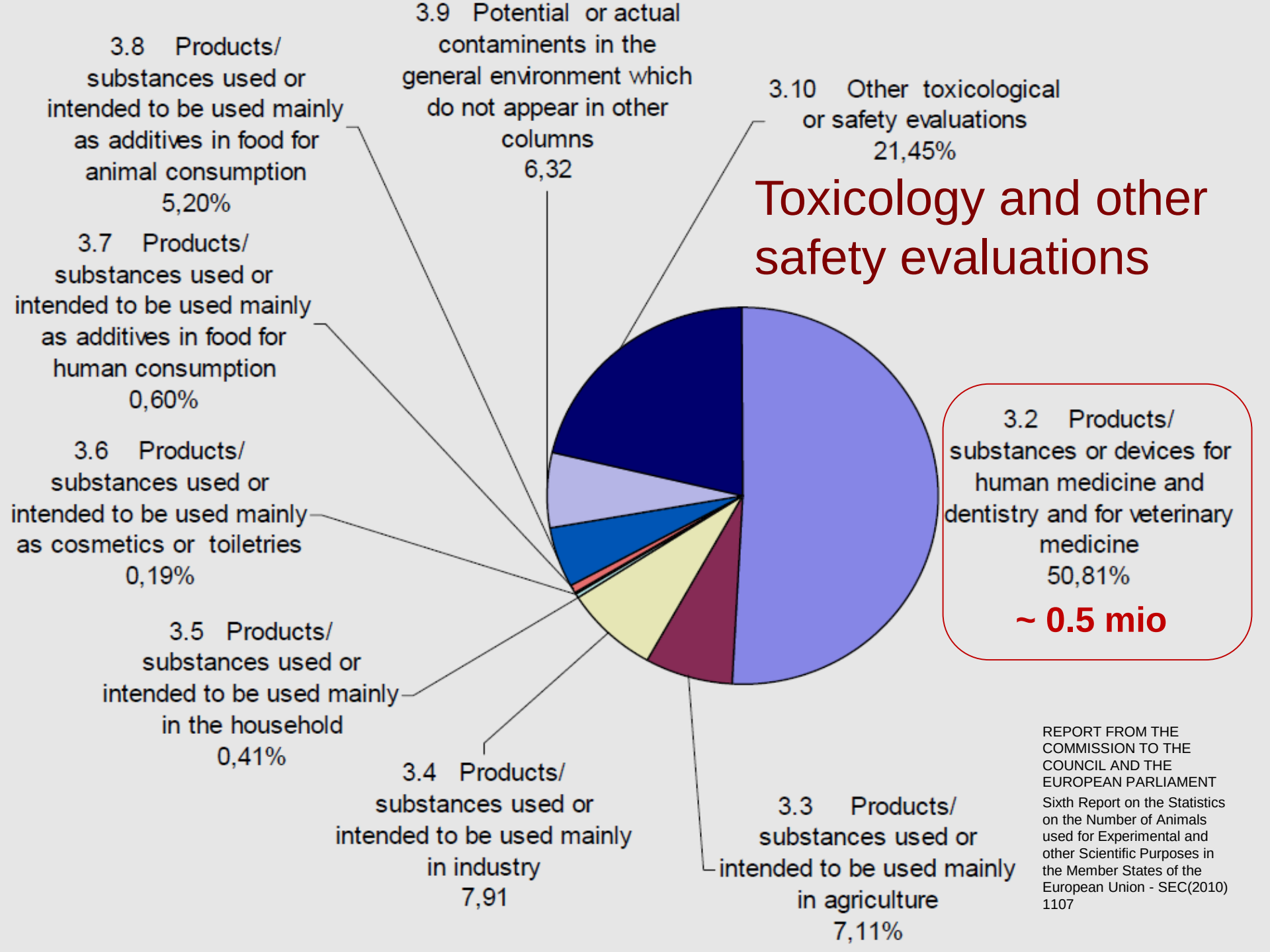
Animals used in 2008 in the EU



REPORT FROM THE COMMISSION TO THE COUNCIL AND THE EUROPEAN PARLIAMENT

Sixth Report on the Statistics on the Number of Animals used for Experimental and other Scientific Purposes in the Member States of the European Union - SEC(2010) 1107

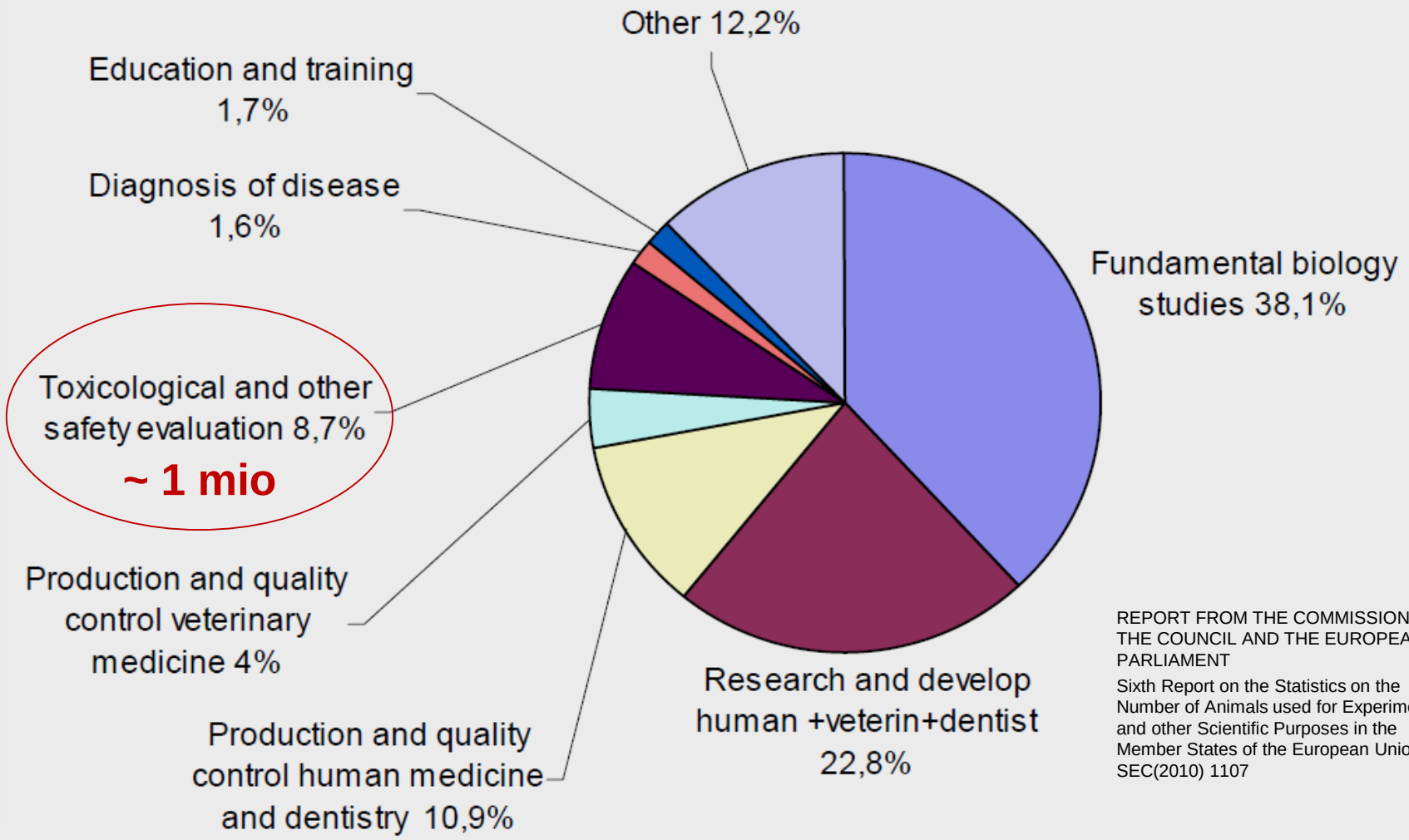
Toxicology and other safety evaluations



REPORT FROM THE COMMISSION TO THE COUNCIL AND THE EUROPEAN PARLIAMENT

Sixth Report on the Statistics on the Number of Animals used for Experimental and other Scientific Purposes in the Member States of the European Union - SEC(2010) 1107

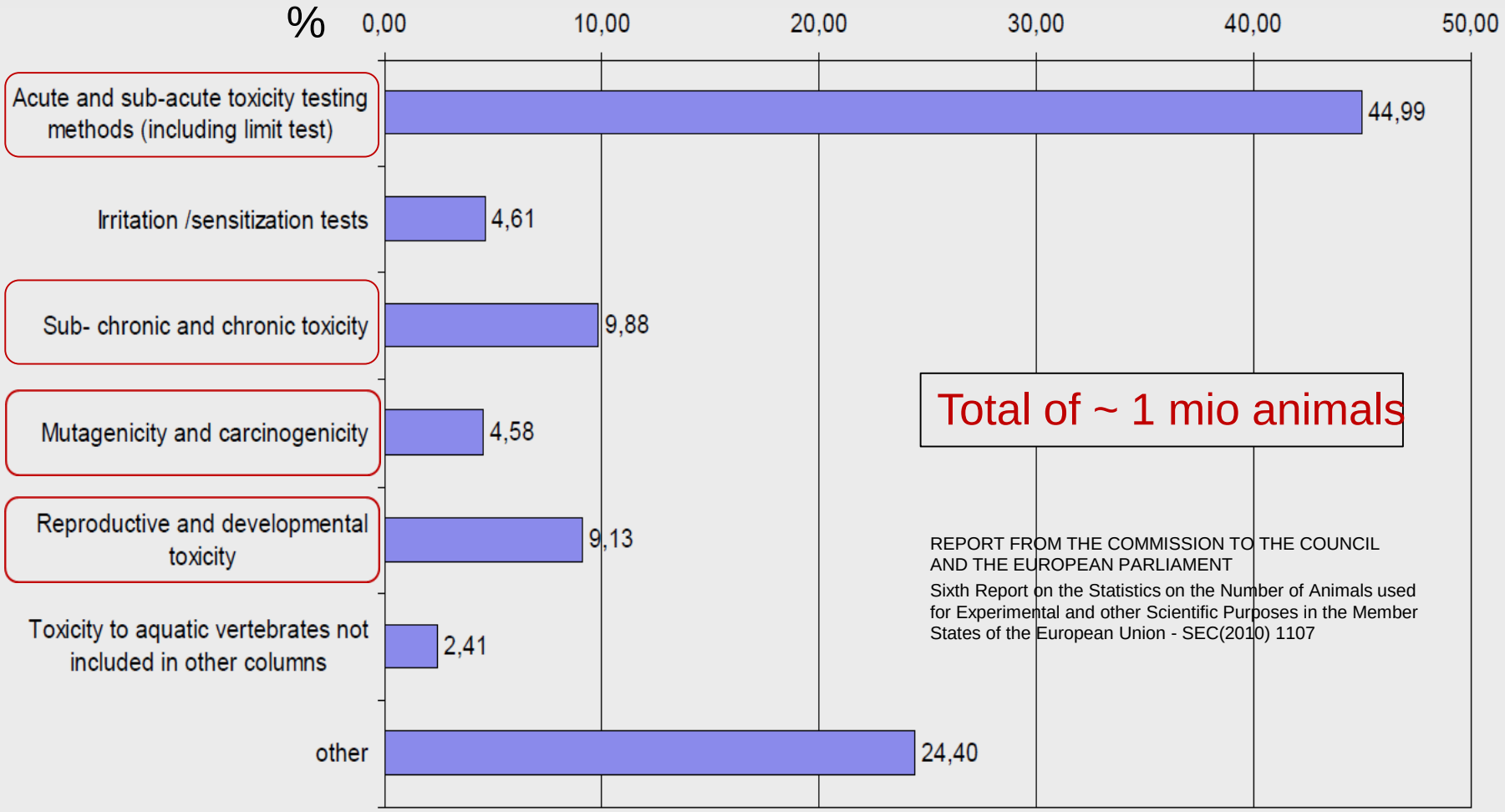
Purposes for using experimental animals



REPORT FROM THE COMMISSION TO THE COUNCIL AND THE EUROPEAN PARLIAMENT

Sixth Report on the Statistics on the Number of Animals used for Experimental and other Scientific Purposes in the Member States of the European Union - SEC(2010) 1107

Type of safety testing



Animal species commonly used in regulatory toxicology

Study type	Primary species	Alternatives
Acute toxicity*	Rodents (rat, mouse)	
Multidose toxicity	Rodent (especially rat) Non-rodent (esp. dog)	Mouse, monkey, minipig
Carcinogenicity	Rodents (rat, mouse)	
In vivo mutagenicity	Mouse	
Development / reproductive tox	Rat, rabbit	Mouse, hamster, monkey
Immunotoxicity	Mouse, guinea pig	Rat

* Today done only for specific reasons

Rodent strains frequently used in toxicology studies

Species	Strain	Characteristics (depending on breeder)
Rat	SD	Outbred, albino, **large database** Can easily be made obese Tumors (especially mammary, thyroid, pituitary, etc.), age-related degenerations (especially kidney)
	Wistar	Outbred, albino, **good as 2 years survival** Tumors (especially mammary, thyroid, etc.)
	Fischer 344	Inbred, albino, **small** Tumors (especially leukemia, pituitary, thyroid, etc.)
Mouse	CD-1	Outbred, albino Tumors (especially liver), amyloidosis
	C57BL	Inbred, **black**
	BLAB/c	Inbred, albino, testicular atrophy

Overview

- Use of animals
- Regulations regarding species selection
  - General and carcinogenicity testing
  - Reproductive toxicity testing
  - Testing of biotechnology products
- Species similarities and differences
- Commonly used species in toxicology
- Humans
- Adverse drug reactions in humans
- Extrapolation of animal data
- Species selection
- Conclusions

M4S (R2): Common Technical Document
Nonclinical Summaries and Organization of Module 4

Mouse
Rat
Hamster
Other rodent
Rabbit
Dog
Non-human primate
Other non-rodent mammal
Non-mammals

General statement in regulations

- Use the relevant species
- ICH M3 (R2)
  Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals
  - 2 mammals, of which one non-rodent
- ICH M4S (R2)
  CTD: Nonclinical Summaries and Organization / Mod 4
  - Species- or gender-related differences
  - Interspecies comparison of metabolism

S1B Testing for *carcinogenicity* of pharmaceuticals

4.2.1 Choice of species for a long-term carcinogenicity study

The species selected should be appropriate, based on considerations that include the following:

(a) Pharmacology

(b) Repeated-dose toxicology

(c) Metabolism (see also Guidelines S1C and S3A)

(d) Toxicokinetics)TK) (see also Guidelines S1C, S3A, S3B)

(e) Route of administration

In the absence of clear evidence favoring one species, it is recommended to select the rat

S5 (R2): Detection of toxicity to *reproduction and male fertility*

- Studies should be conducted in *mammalian species…*
  Reasons for using *rats* as the predominant rodent species are
  - Practicality
  - Comparability with results from toxicity studies
  - Large amount of background knowledge
- Embryotoxicity testing
  A *second mammalian species* generally required, the rabbit preferred as "non-rodent“, because of
  - Extensive background knowledge
  - Availability
  - Practicality

S5 (R2): Note 5 (2.1) Selection of species and strains - 2

All species have their disadvantages, for example:

- *Rats:* susceptibility to *sexual hormones;* unsuitable for *dopamine agonists* due to dependence on prolactin as the primary hormone for early pregnancy; highly susceptible to *non-steroidal anti-inflammatory drugs* in late pregnancy
- *Mice:* fast metabolic rate; stress sensitivity; malformation clusters (which occur in all species) particularly evident; small fetuses
- *Rabbits:* susceptibility to some *antibiotics* and to disturbance of the alimentary tract; clinical signs can be difficult to interpret; often lack of kinetic and toxicity data
- *Guinea pigs:* susceptibility to some *antibiotics* and to disturbance of the alimentary tract; long fetal period; often lack of kinetic and toxicity data; insufficient historical background data

S5 (R2): Note 5 (2.1) Selection of species and strains - 3

Continued: All species have their disadvantages, for example:

- Domestic and/or *minipigs:* malformation clusters with variable background rate; large (compound, housing requirements), insufficient historical background data
- *Ferrets:* seasonal breeder unless special management systems used; insufficient historical background data
- *Hamsters:* sensitive to intestinal disturbance; overly sensitive teratogenic response to many chemicals; small fetuses, experimental difficulties (e.g. intravenous i.v. route difficult; can hide doses in cheek pouches; can be very aggressive)
- *Dogs:* variable breeders; inbreeding factors; insufficient historical background data also regarding reproduction
- *Non-human primates:* PK can differ from humans as in other species; insufficient historical background data; often the numbers used are too low

S6 (R1): *Biotechnology*-derived pharmaceuticals - 1

Appropriate nonclinical species based on:

- Binding to
  - Target: *in vitro* receptor binding studies
  - Off-target: tissue cross reactivity
- Pharmacodynamics PD (*binding ≠ activity*)
  An animal species without the desired epitope may still be of some relevance in case of *comparable tissue cross-reactivity*

→ Non-human primates (NHP) often the only relevant species

S6 (R1): Biotechnology-derived pharmaceuticals - 2

Toxicity studies in non-relevant species are discouraged.

If

- *No relevant species* can be identified or
- For *studies difficult* to perform in NHP, such as carcinogenicity and reproductive toxicity tests

Consider the use of:

- *Homologous (surrogate) test protein*
  Potential problems: may not be predictive, impurities and contaminants, cost and time
- *Transgenic animals* with the human receptor or epitope

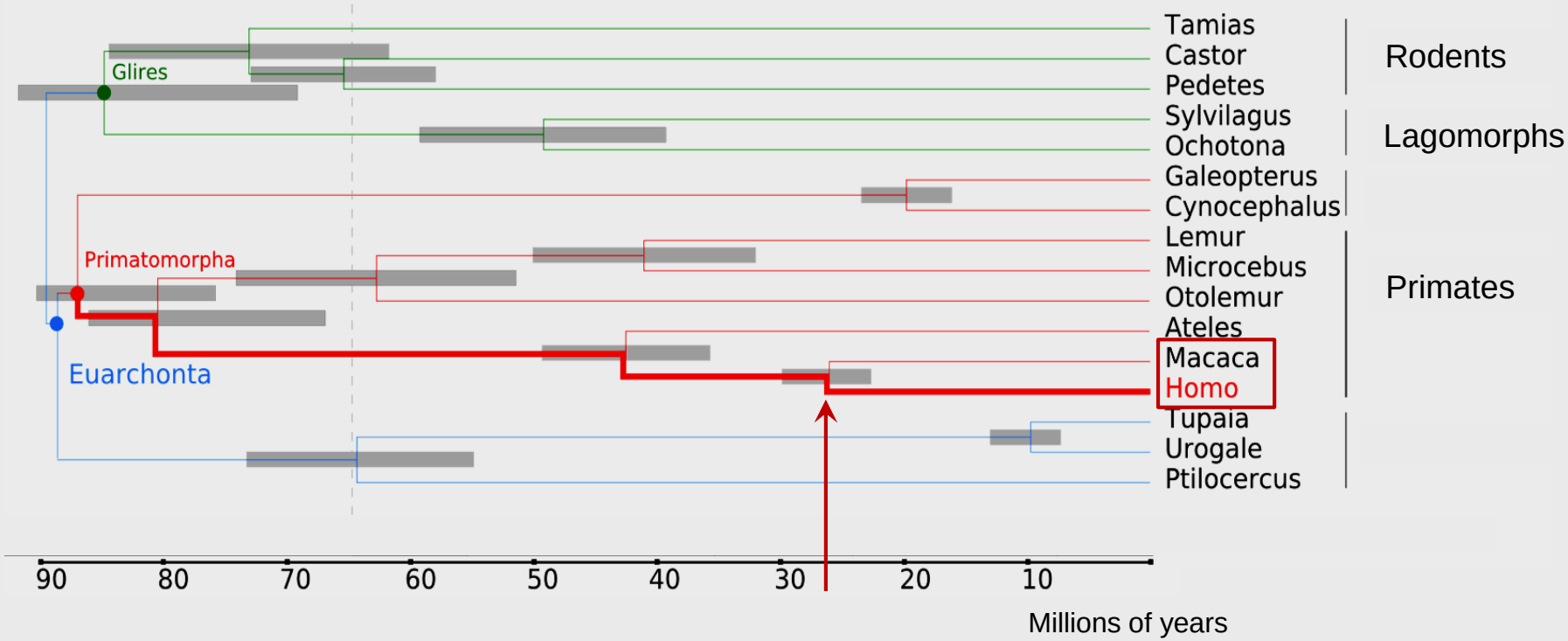
Overview

- Use of animals
- Regulations regarding species selection
- Species similarities and differences
  - General
  - Anatomy
  - Physiology and biological behavior
  - Receptors and epitopes
  - Toxicokinetics
- Commonly used species in toxicology
- Humans
- Adverse drug reactions in humans
- Extrapolation of animal data
- Species selection
- Conclusions

Millions of years ...

Wikipedia http://de.wikipedia.org/wiki/Stammesgeschichte_des_Menschen. Accessed in January 2021

… separate humans from test animals



Species differences – Number of articles

Journal	Species differences	# articles in total	Since
Tox Path	32	2580	1983
J Tox Path	56	> 500	1998
Exp Tox Path	558	1566	1992

Number of articles based on searches in corresponding website in 2012

Total number of articles based on

- Searches in PubMed for Tox Path and Exp Tox Path
- Estimate for J Tox Path (not in PubMed)

Prediction / extrapolation – Number of articles

Journal	Prediction	Extrapo-lation	# articles in total	Since
Tox Path	222	249	2580	1983
J Tox Path	18	12	> 500	1998
Exp Tox Path	76	70	1566	1992

Number of articles based on searches in corresponding website in 2012

Total number of articles based on

- Searches in PubMed for Tox Path and Exp Tox Path
- Estimate for J Tox Path

Animal models useful?

Is the glass

- ☐ Half-full

or

- ☐ Half-empty?

Animal models useful?

Is the glass

- ☐ Half-full

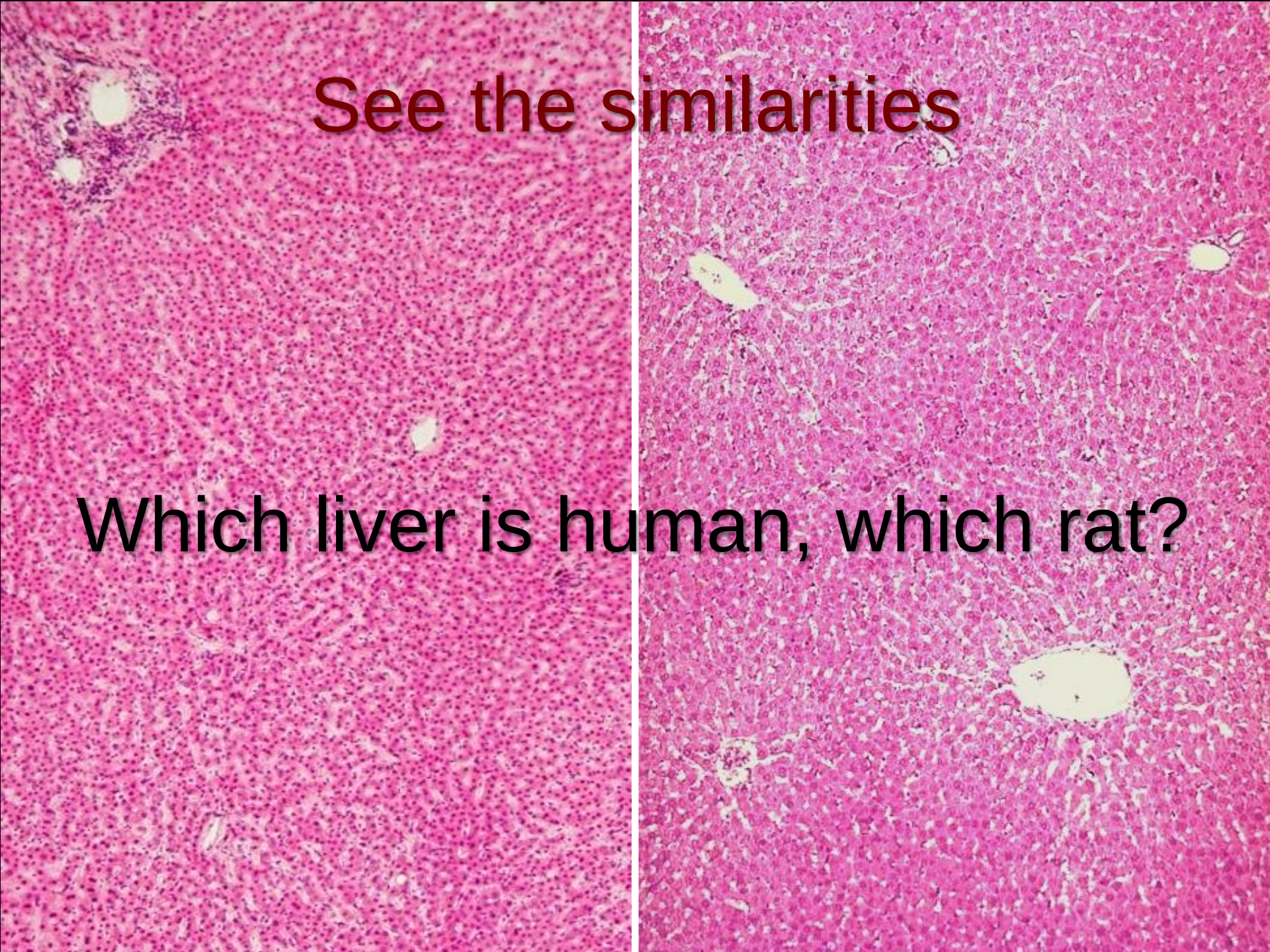
Or

- ☐ Half-empty?

See the similarities

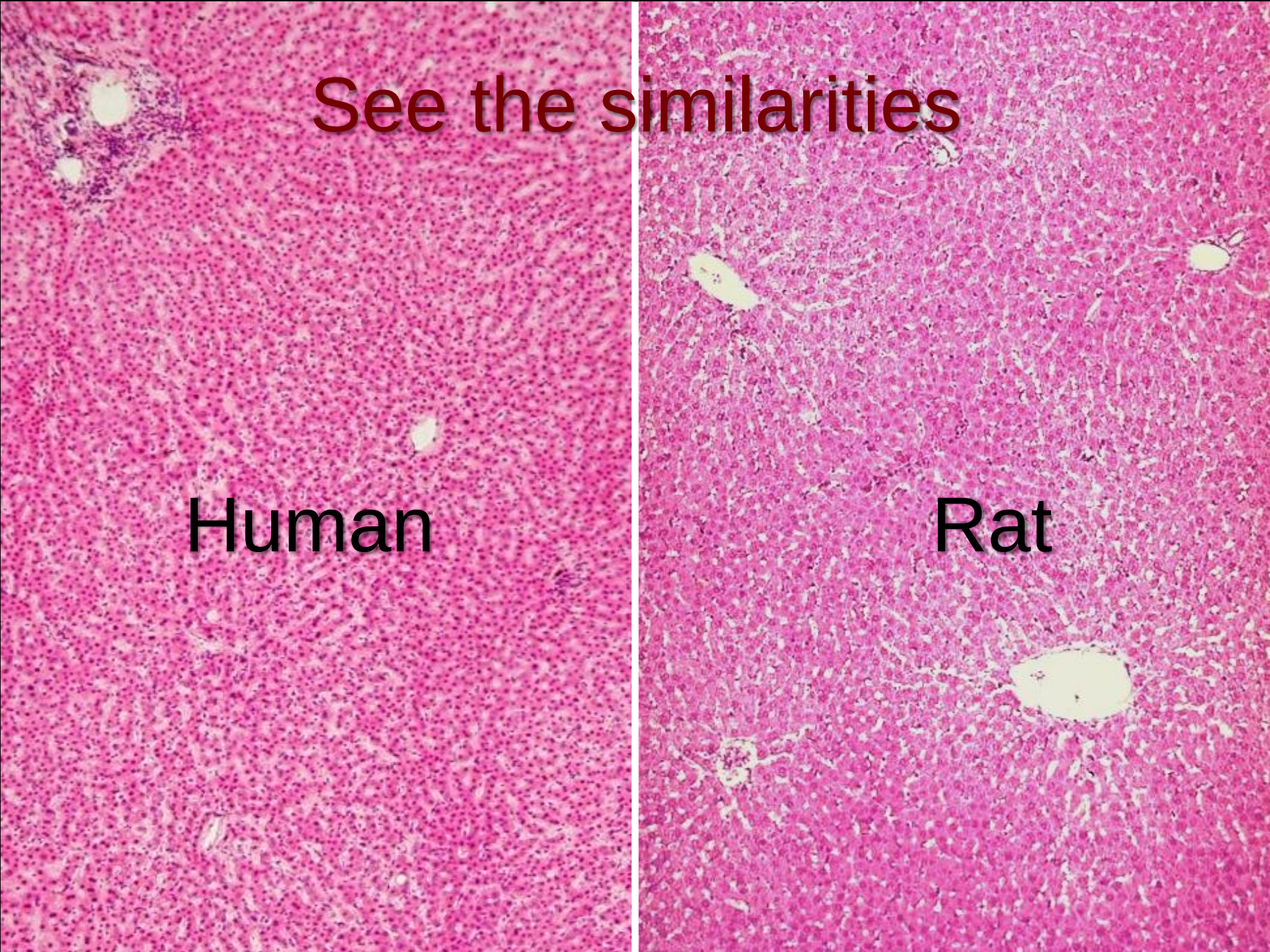
Understand the differences

See the similarities



Which liver is human, which rat?

See the similarities



Understand the differences

Species differ with regard to:

1. Anatomy
2. Physiology (especially hormones) and biological behavior (especially tumors)
3. Receptors and epitopes of relevance for toxicity, including pharmacokinetics (PK) and PD, especially for biotherapeutics
4. TK (ADME)
5. Etc. such as “sensitivity”

Animal size influences …

- Body surface area:
  Temperature control and water management
- Doubling of the bodyweight
  → 180% ↑ metabolic rate
  → 185% ↑ life span
- Sample size and number:
  Biochemical analysis, organs
- Manipulations
- Drug requirements (also critical: group size)

What is an equivalent dose?

Species	Weight kg	Surface area cm^2	Dose in mg per …		
			kg bw	Individual	Surface cm^2
Mouse	0.020	46	*100*	2	0.043
Rat	0.200	325	*100*	20	0.061
Dog	12.000	5770	*100*	1200	0.207
Human	70.000	18000	*100*	7000	0.388

http://toxicology.usu.edu/660/html/testing.htm
Website no longer accessible

Test material for various species

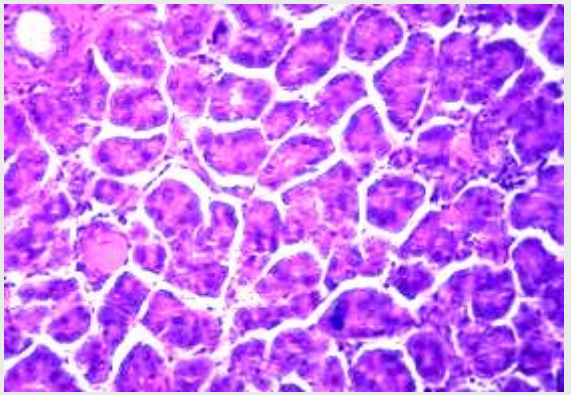
	Mouse	Rat	Dog	Cynomolgus
Body weight (BW)	30 g	250 g	9 kg	2.5 kg
Test group size (#/sex)	10	10	4	4
Test material (approx., g)	~ 45	~ 360	~ 5100	~ 1420

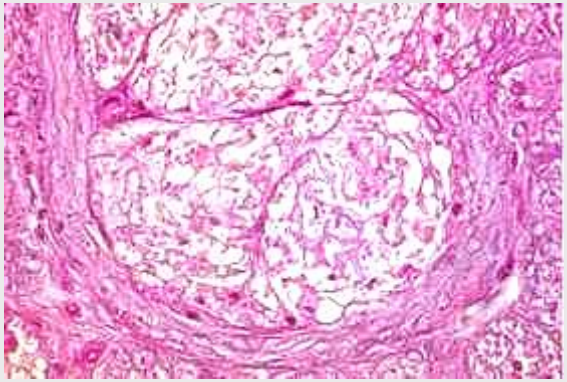
28 day study
4 test groups at doses of 0, 25, 100 and 400 mg/kg/d
+ 20% spare material

After Toxicology Testing Handbook 'Principles, Applications, and Data Interpretation' Editors. D. Jacobson-Kram and K. A. Keller - CRC Press; 2nd edition

Anatomical particularities of rodents

- *Forestomach*
  With squamous epithelium
- *Harderian gland*
  Accessory tear gland for 3rd eye lid
- *Zimbal's gland*
  Sebaceous gland around external ear canal
- *Preputial gland*
  Similar to sebaceous gland





Particularities of the rodent endocrine system

- Less serum *binding* of hormones
- *Estrogen/progesterone ratio* 1:100(-200) in rodents vs. 1:1 in women
- *Different senescence:* progesterone dominance in old rats vs. waning system in women
- *Prolactin* with trophic effect on rat mammary gland vs. lactation maintaining effect in women
- *High susceptible to disturbance* of hormonal regulation, e.g. luteinizing hormone (LH) leads to hyperplastic/“neoplastic” Leydig cells (LC; not in man: Klinefelter)
- Etc.

Reproductive parameters in various species

Species	Puberty	Sexual Cycle	Gestation length	Litter size	Litters / year	Age at weaning
MOUSE	6-8 (9) wks	4-7 (5) days	19-20 days	6-10 (8)	4-8 (5)	19-21 days
RAT	7-11 (12) wks	3-8 (5) days	21-23 (22) days	6-10	5-6	20-22 days
HAMSTER	4-6 (8-9) wks	4-7 (4) days	15-17 (169) days	16 (5-8)	5-6	3-4 weeks
GUINEA PIG	F: 4 wks M: 8-9 wks	13-20 (14-16) days	59-74 (63) days	1-7 (3-4)	5	2 weeks
RABBIT	4-5 (9) months	14-16 days	28-36 (30-33) days	1-16 (7-8)	5-6	6-8 weeks
FERRET	9-12 months	seasonal	42 days	6-10	1-2	8 weeks
DOG	9-15 months	3 weeks, twice/year	58 - 66 (63) days	3-8	1.5 - 2	6-8 weeks

After: Chapter 13: The Biology of Laboratory Animals - A Hem
Laboratory Animal Science: A Hem, D M Eide, E Engh and A Smith (eds)
January 2001, ISBN 82-7725-117-3. Partly revised in September 2010

Mean bile flow

Species	Bile flow (µL/min/kg BW)
Mouse	78
Rat	30 - 150
Guinea pig	200
Rabbit	90
Pig	9
Dog	4 – 10
Monkey	10
Human	1.5 - 15

Martinez M et al Adv Drug Deliv Rev 2002, 54:825-850

Glomerular filtration rates

Species	ml/min/kg BW
Mouse	10
Rat	8.7
Rabbit	4.8
Dog	4
Monkey	2
Human	1.8

Tibbitts J. Toxicol Pathol 2003, 31 Suppl:17-24

Serum proteins (g/dl)

	Rat	Rabbit	Dog	Human
Total protein	6.5	7.0	5.8	7.8
Albumin	2.1	4	3.5	4.9

Tibbitts J Toxicol Pathol 2003, 31 Suppl:17-24

Tumor biology in rodents

- High *spontaneous* incidence in endocrine and some other organs (liver, lung, hematopoietic system, etc.)
- Often at *multiple* sites
- *Easy to induce*, e.g. by subcutaneous irritation, including e.g. foreign body reaction
- Tend to be *less aggressive/malignant*, e.g. few metastases

Factors influencing lung toxicity

Animal	% identity with human HVR TLR4	LPS sensitivity	Intravascular macrophages	Nitric oxide production
Mouse	48	Low	No	+++
Rat	48	Low	No	+++
Rabbit	57	Intermediate	No	++
Dog	*n.a.*	Low	No	++
Pig	*n.a.*	High	Yes	++
NHP	95	Intermediate	No	+
Human	100	Intermediate	No	+

HVR TLR4 : Hypervariable region of Toll-like receptor 4
LPS: Lipopolysaccharide
n.a. : *Not available*

Animal models of acute lung injury. Matute-Bello G, Frevert CW, Martin TR. J Physiol Lung Cell Mol Physiol 2008, 295(3): L379-99

Family	Subfamily	Humans	Mouse	Rat	Dog	Monkey
CYP1	A	1A1, 1A2	1A1, 1A2	1A1, 1A2	1A1, 1A2	1A1, 1A2
	B	1B1	1B1	1B1	1B1	1B1
CYP2	A	2A6, 2A7, 2A13	2A4, 2A5, 2A12, 2A22	2A1, 2A2, 2A3	2A13, 2A25	2A23, 2A24
	B	2B6, 2B7	2B9, 2B10	2B1, 2B2, 2B3	2B11	2B17
	C	2C8, 2C9, 2C18, 2C19	2C29, 2C37, 2C38, 2C39, 2C40, 2C44, 2C50, 2C54, 2C55	2C6, 2C7*, 2C11*, 2C12*, 2C13*, 2C22, 2C23	2C21, 2C41	2C20, 2C43
	D	2D6, 2D7, 2D8	2D9, 2D10, 2D11, 2D12, 2D13, 2D22, 2D26, 2D34, 2D40	2D1, 2D2, 2D3, 2D4, 2D5, 2D18	2D15	2D17* 2D19* 2D29* 2D30* 2D42*
	E	2E1	2E1	2E1	2E1	2E1
3	A	3A4, 3A5, 3A7, 3A43	3A11, 3A13, 3A16, 3A25, 3A41, 3A44	3A1/3A23, 3A2*, 3A9*, 3A18*, 3A62	3A12, 3A26	3A8

Enzyme diversity of the major CYP families

Thesis 2006:
Species and strain differences in drug metabolism in liver and intestine
M Martignoni
ISBN: 9036727138

Some species-specific metabolic deficiencies

- *Rat:* deficiency in the N-hydroxylation of aliphatic amines
- *Dog:* inability to acetylate aromatic amines
- *Guinea pig:* deficiency in N-acetylation and unable to form N-acetylate-S-substituted cysteines
- *Pig:* deficiency in most sulfation reactions

Interspecies Differences in Physiology and Pharmacology: Extrapolating Preclinical Data to Human Populations
M. N. Martinez
In: Preclinical drug development. Mark C. Rogge, David R. Taft (eds) 2nd ed. 2010, pp 35-70
Informa Healthcare USA, Inc.

Phenol conjugation (phase 2 reactions)

	Sulfate (S)	Glucuronic acid (G)	Ratio S/G
Human Old world monkeys	80	12	7
New world monkeys	25	50	0.5
Rat / Mouse	45	40	1.13
Pig	2	95	0.02

V. Beasley. International Veterinary Information Service (www.ivis.org), Ithaca, NY, USA
No longer accessible under http://www.ivis.org/advances/beasley/appc/ivis.pdf

Free fraction of drugs in blood

Drug	Mouse	Rat	Dog	Monkey	Human
Cefpiramide	0.56	0.54	0.70	0.068	0.037
Cefoperazone	0.854	0.744	0.744	0.161	0.176
Cefmetazole	0.65	0.56	0.75	0.19	0.15
Diazepam		0.137	0.04		0.032
Quinidine	0.363	0.324			0.13
Valproic acid	0.881	0.366	0.215		0.052
Meloxicam	0.04	0.003			0.004
CIPB	0.65	0.25	0.15	0.05	0.03
Etodolac	0.052	0.007	0.017	0.012	0.008
Tolrestat	0.04	0.017	0.02	0.014	0.007
Pelrinone	0.78	0.28	0.20	0.21	0.11
Benoxaprofen	0.011	0.007	0.008	0.004	0.002

After: Cayen MN *in* Human Risk Assessment: The Role of Animal Selection and Extrapolation. Philadelphia, PA: Taylor & Francis / Mahmood I. J Clin Pharmacol 2000 40:1439-1446

NSAID Plasma half-life in 4 species

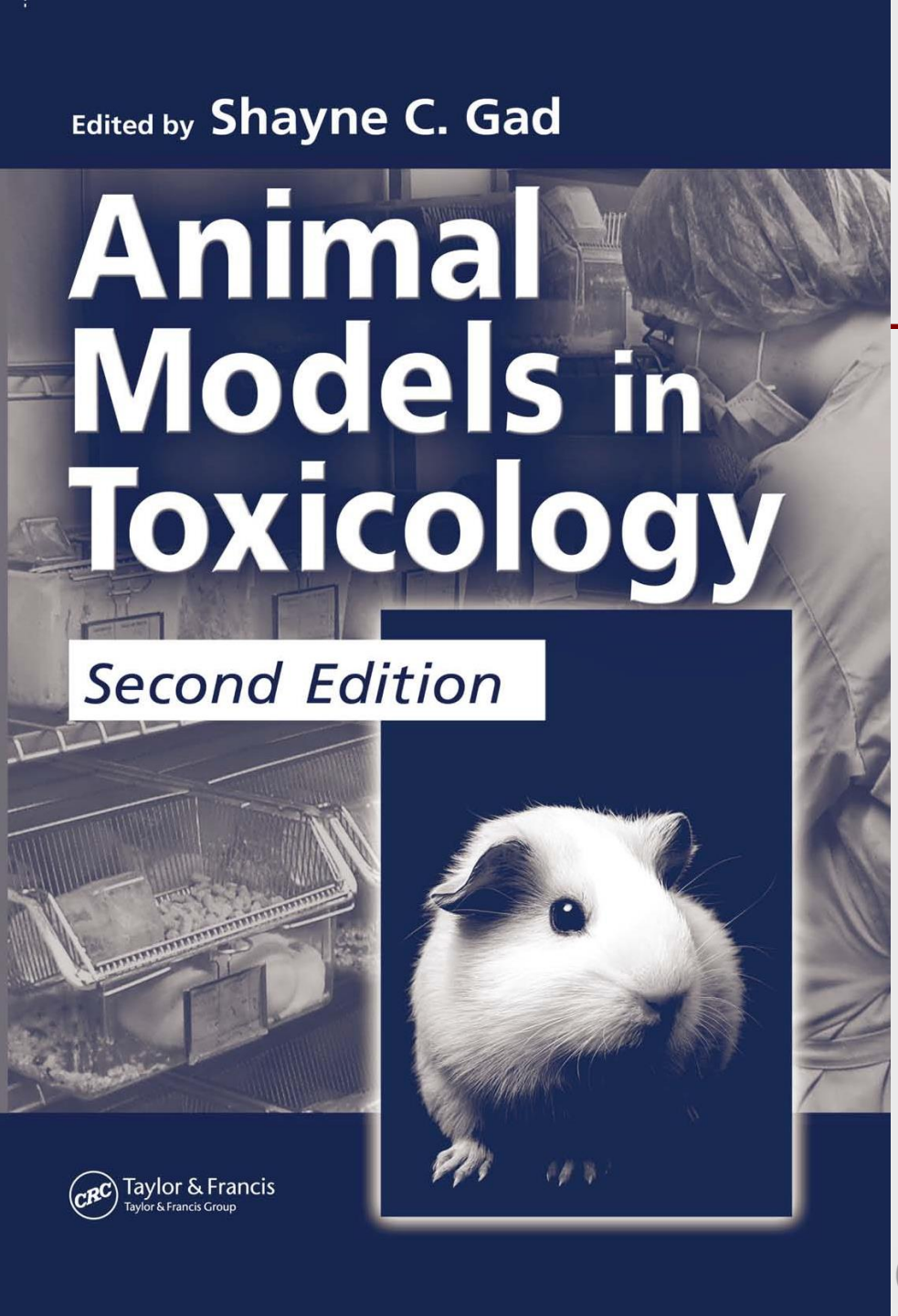
NSAID	Rat	Dog	Monkey*	Human
Piroxicam	6 (m), 16 (f)	*45*	5	*45*
Indomethacin	*4*	0.3	0.3	*2*
Naproxen	5	*35*	1.9	*13.9*
Ibuprofen	1	*2.5*		*3*
Phenylbutazone	6	6	7	*72*
Fenprofen	8	*4*	0.3	*2.5*
Sulindac	4			*8*

* No details regarding species

V. Beasley. International Veterinary Information Service (www.ivis.org), Ithaca, NY, USA
No longer accessible under http://www.ivis.org/advances/beasley/appc/ivis.pdf

Overview

- Use of animals
- Regulations regarding species selection
- Species similarities and differences
- Commonly used species in toxicology
  - Rodents: Rat, mouse, hamster, guinea pig
  - Non-rodents: rabbit, ferret, dog, pig, monkey
- Humans
- Adverse drug reactions in humans
- Extrapolation of animal data
- Species selection
- Conclusions



Information on animal models

Animal Models in Toxicology

2nd edition, 2007

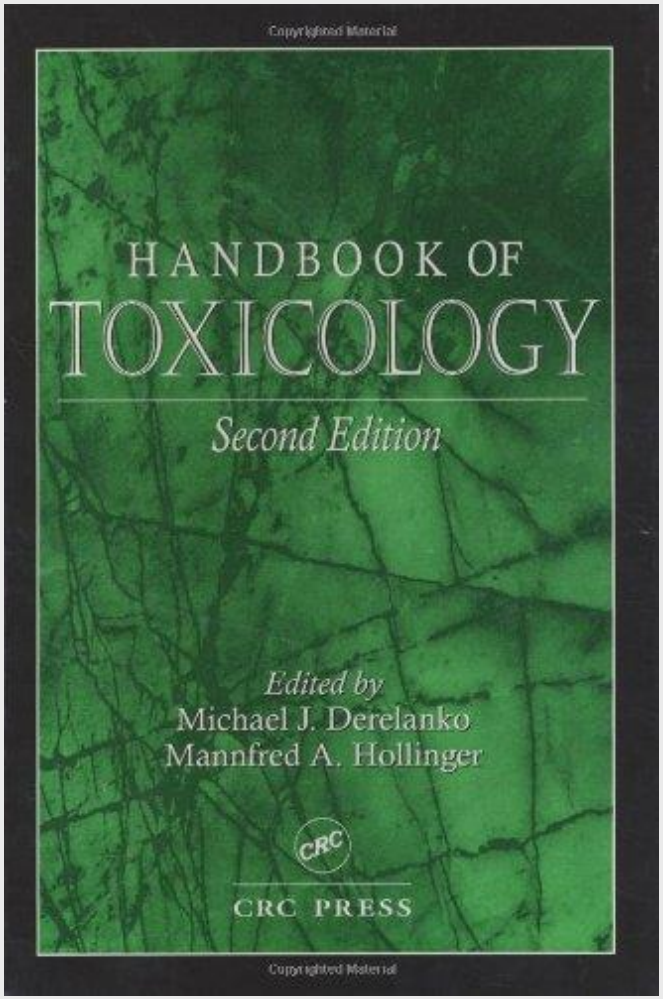
Shayne C. Gad (editor)

Taylor & Francis Group

Boca Raton London New York

Also available as e-book

Handbook of Toxicology



Handbook of Toxicology

Michael J Derelanko, Mannfred A Hollinger

Taylor & Francis Inc

CRC Press 2002

Especially Chapter 1 “Laboratory Animal Management”

Caveat

- Within one species significant differences can exist between different *strains*, different *breeders* and different *laboratories* (husbandry, etc.), etc.
- *Comparisons* of figures and statements from different sources need to be done with caution: methods, circadian rhythms, etc.

Addressed for each species are …

- Some biological key parameters in comparison with humans
- If appropriate:
  - Comparison with alternative species
  - Particular use
- Some characteristics including advantages and disadvantages
- For common spontaneous lesions (depend on many variables: *see appendix* (for illustration and not comprehensive)

Biology	**Rat**	***Human***
Weight	100-700 gr	*60-110 kg*
Life span	2.5-3 years	*60-90 years*
O_2 consumption	0.76 ml/g/hr	*0.4 ml/g/hr*
Heart rate	300-390/min	*60-90/min*
BP	125/85 mmHg	*120/80 mmHg*
Puberty	40-60 days	*12-16 years*
Gestation	21-23 days	*40 weeks*
Weaning	21 days	*> 6 months*
Estrus cycle	4-5 days	*28 days*

Rat (also valid for the mouse)

Advantages

Commonly used → large historic database
Small size
- Minimal housing space

Prolific
- Short gestation
- Short lactation
- Short life span

Omnivorous
- Dry diet acceptable

Dosing by multiple routes
No emetic reflex
Inexpensive
- Low maintenance cost

Docile
Intelligence
Often similar metabolism of xenobiotics

Disadvantages

Anatomic differences
- ☐ Lack of gallbladder (only rats)
- ☐ Yolk-sac placenta
- ☐ Multiple mammae over body surface
- ☐ Fur covered
- ☐ Thinner stratum corneum
- ☐ No bronchial glands

Physiological differences
- ☐ Estrus and menstrual cycles
- ☐ Multiparous
- ☐ Hematology
- ☐ Different intestinal flora
- ☐ High metabolic rate (especially mice)
- ☐ Obligatory nose breather
- ☐ Concentrated urine
- ☐ Limited hypersensitivity response

After Animal Models in Toxicology, 2nd edition, 2007, Shayne C. Gad (editor). Taylor & Francis Group, Boca Raton London New York, P 907

Rat (also valid for the mouse)

Disadvantages

Aging

- Degenerative lesions
- Proliferative lesions (prone also for induced proliferations

Metabolic differences

- Metabolism of xenobiotics
- Purines to allantoin
- High β-glucuronidase activity

Nutritional differences

- Mineral requirements
- Vitamin requirements
- Ascorbic acid biosynthesis
- Histidine biosynthesis

Disadvantages

Behavioral differences

- Nocturnal
- Coprophagy
- Cannibalism

Specific maintenance requirements

- Temperature and humidity control
  Difference in thermoregulation
- Noise control

Strain differences

Calabrese E J
Principles of animal extrapolation Wiley, New York, 1983

Biology	**Mouse**	***Human***
Weight	20-40 gr	*60-110 kg*
Life span	1-3 years	*60-90 years*
O_2 consumption	1.69 ml/g/hr	*0.4 ml/g/hr*
Heart rate	320-800/min	*60-90/min*
BP	145/105	*120/80 mmHg*
Puberty	50-60 days	*12-16 years*
Gestation	17-21 days	*40 weeks*
Weaning	16-21 days	*> 6 months*
Estrus cycle	4-5 days	*28 days*

Mice vs. rats

- *Very small*
  - ➔ Low compound requirement
    May limit experimental procedures
  - Very high metabolic rate ("fast ADME")
  - Less easy to handle than rats
- Availability of *genetically modified mice*
- Urinary excretion
  - High glomerular filtering surface per bw (> rat)
  - Urine about four times as concentrated as the highest human concentrations
- Stressed by noise
- Single housing good, but more expensive

Biology	**Hamster**	***Human***
Life span	2-3 years	*60-90 years*
Weight	100-135 gr	*60-110 kg*
O_2 consumption	2.3 ml/g/hr	*0.4 ml/g/hr*
Heart rate	300-600/min	*60-90/min*
BP	Systolic 110 mmHg	*120/80 mmHg*
Puberty	4-6 (f), 6-7 (m) weeks	*12-16 years*
Gestation	16 days	*40 weeks*
Weaning	21-28 days	*> 6 months*
Estrus cycle	4 days	*28 days*

Hamsters

- Strain
  - > 80% Syrian hamsters
  - < 20% Chinese hamsters
- Formerly used for carcinogenicity testing because of *low spontaneous tumor* incidence and relatively short life span (as other rodents)
- Preferably single housing

Biology	Guinea pig	*Human*
Weight	600-1000 g	*60-110 kg*
Life span	2-6 years	*60-90 years*
O_2 consumption	0.76 ml/g/hr	*0.4 ml/g/hr*
Heart rate	240-280/min	*60-90/min*
BP	75/45 mmHg	*120/80 mmHg*
Puberty	45-70 days	*12-16 years*
Gestation	59-70 days	*40 weeks*
Weaning	21-28 days	*> 6 months*
Estrus cycle	16-18 days	*28 days*

Guinea pigs

- Relatively expensive
- Small, easy to handle, but *susceptible to noise* (might refuse to drink and eat)
- No readily accessible peripheral veins for i.v. injections and blood collection
- Most commonly used: short-haired English variety, especially Hartley strain
- Relatively new: *hairless strain* (Charles River Laboratories) for dermal studies

Main use: Immunology

- Immediate type hypersensitization (systemic immune response often exaggerated)
- Delayed type dermal sensitization
- Photosensitization
- Pulmonary sensitization
- Host-resistant assay (instead of mice)

Further uses – 1

- Audiology including *ototoxicity* caused e.g. by NSAID or antibiotics
- *Ocular toxicity:* cataractogenesis by dermal and oral application
- *Infectious diseases* (sensitive to human tuberculosis)
  Colitis model (induction by carrageenan)
- *Teratology*
- *Inhalation* toxicology

Further uses – 2

- Carcinogenicity testing
  - Low incidence of spontaneous neoplasms
  - Compared to rats relatively insensitive to certain carcinogens such as aromatic amines (low metabolic activation?)

Biology	**Rabbit**	***Human***
Weight	4-6 kg	*60-110 kg*
Life span	5-13 years	*60-90 years*
O_2 consumption	0.5-0.85 ml/g/hr	*0.4 ml/g/hr*
Heart rate	120-280/min	*60-90/min*
BP	110/75 mmHg	*120/80 mmHg*
Puberty	3-8 months	*12-16 years*
Gestation	29-35 days	*40 weeks*
Weaning	4-6 weeks	*> 6 months*
Estrus cycle	Induced	*28 days*

Rabbits – 1

- Relatively inexpensive (compared to cats, dogs, and monkeys)
- Easy to house and handle
- Small
- Hardy, clean
- SPF rabbits difficult/impossible to obtain
  - Often subclinical infections with seasonal variation
  - Pasteurella infections frequent in various organs

Rabbits – 2

- Intestinal microflora more like in men (compared to rodents and guinea pigs), but easily disturbed (→ diarrhea, etc.)
- Very sensitive to certain antibiotics
- Very variable gastric emptying (20 min – 20 hours!)
  → for oral studies not well suited
- Sensitive to heat

Rabbits – Use

- Teratology
  - Relatively sensitive to teratogens
  - Short gestation (similar to rodents)
  - Fetuses large enough for examination
- Immunology: good antibody production
- Various other studies including
  - Dermal, mucosal
  - Ocular
  - Implant testing

Rabbits: Dermal studies

In comparison to human skin: thinner stratum corneum

→ Higher dermal absorption

→ Shorter exposure of skin surface, but temporarily higher concentrations within rabbit skin

→ Systemic exposure markedly different from that of men

Biology	**Ferret**	***Human***
Weight	800-1600 gr	*60-110 kg*
Life span	5-8 years (?)	*60-90 years*
O_2 consumption	(0.7 ml/g/hr)	*0.4 ml/g/hr*
Heart rate	200-380/min	*60-90/min*
BP	150/115 mmHg	*120/80 mmHg*
Puberty	8-10 weeks	*12-16 years*
Gestation	39-46 days	*40 weeks*
Weaning	6 weeks	*> 6 months*
Estrus cycle	Seasonal, induced ovulation	*28 days*

Ferrets – 1

- Small non-rodent species (males up to 1.6 kg, females up to 800 gr)
- Seems to be increasingly used in toxicology (?)
- Particularly in the US also used as pets
  Relatively inexpensive
- Small number of vendors
- Not much experience / historical data available

Ferrets – 2

- Heterogeneity
- “Cleanness” questionable
- For adults individual caging needed
- Sexually inactive at 6-10 hrs light to 18 - 14 hrs dark (simulate early winter)
- Sexually mature at 6 – 8 months

Ferrets – 3

- Cave heat stress particularly during transport
  Can lead to stress-related gastric ulcers
- Infections: prone for influenza and pneumonitis (can be used as model for influenza and influenza vaccine testing)
- Estrus-associated aplastic anemia. Can be lethal because of hemorrhage

Biology	Dogs	*Human*
Weight	10-12 kg	*60-110 kg*
Life span	12.5 years	*60-90 years*
O_2 consumption	0.36 ml/g/hr	*0.4 ml/g/hr*
Heart rate	120-150/min	*60-90/min*
BP	120/60 mmHg	*120/80 mmHg*
Puberty	6-12 months	*12-16 years*
Gestation	60.65 days	*40 weeks*
Weaning	5-8 weeks	*> 6 months*
Estrus cycle	Variable (>70-180 days)	*28 days*

Dogs – 1

- Often beagle dogs
- Expensive
- Sometimes short in supply and of “low” quality
- Beagle males up to 12 (+) kg, females up to 11 (+) kg. BW variation

  ➔ Much test compound needed
- Large enough for investigative procedures
- Puberty at 6-12 months of age
- Easy to handle, but noisy

Dogs – 2

- Group housing. Exercise and housing requirements in some countries
- Easy vomiting
- Do not tolerate (well)
  - i.v. injection of Tween 20 and cremophor
    ➔ Histamine release reaction (“allergy”)
  - Morphine-like compounds
  - NSAID (gastric toxicity)
  - Positive inotropic agents (prolonged tachycardia not well tolerated)

Biology	**Minipig**	***Human***
Weight	15-40 kg	*60-110 kg*
Life span	15-17 years	*60-90 years*
O_2 consumption	No data	*0.4 ml/g/hr*
Heart rate	70-85/min	*60-90/min*
BP	100/60 mmHg	*120/80 mmHg*
Puberty	3-5 months	*12-16 years*
Gestation	114 days	*40 weeks*
Weaning	21-28 days	*> 6 months*
Estrus cycle	21 days	*28 days*

Minipig

- Relatively large and expensive
- Sensitive animal model
- Often kept in dog facilities
- Group or single housing
- Restricted feeding advisable
- Easy handling
- Well introduced: Ellegaard Göttingen minipig

In contrast to dogs … - 1

- *Increased heart rate* does not lead to myonecrosis
- Non-steroidal anti-inflammatory drugs *(NSAID)* tolerated
- No *arteriopathy* when administered endothelin receptor agonists
- Female minipigs tolerate *anti-gestagens* and hormones with *estrogenic* activity

In contrast to dogs … - 2

- *Less vomiting* (including vomiting with morphine-like drugs)
- *Less histamine liberation* e.g. with i.v. Tween and cremophor
- Better *acceptance* as experimental animals (not so much used as pets)
- *Earlier sexual maturity*

Comparability with humans

Organ	Similarities with humans, e.g.
GI tract	• Omnivore with comparable teeth • Stomach and small intestine regarding e.g. pH, transit time, cell types and secretions
CV System	• Heart and great vessels • Stress-related diseases
Pulmonary system	• Useful model for respiratory distress syndrome • Nasal and pulmonary structure
Urogenital system	• Kidney: size, number of lobes, and structure • Bladder: good model for urinary incontinence
Eye	Highly developed

For metabolism and skin testing see later
CV = cardiovascular

Metabolism

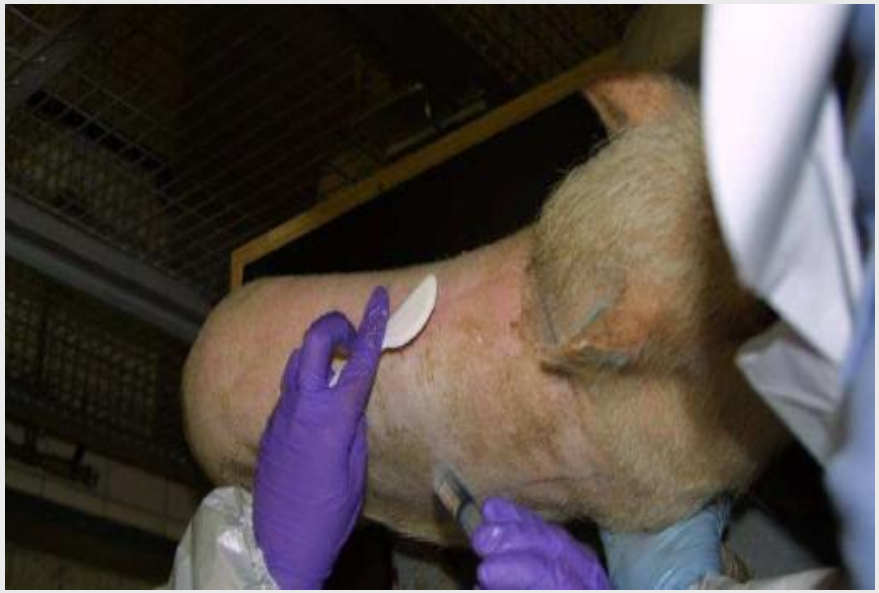
- High conformance with important human isoforms of *cytochrome P450,* particularly CYP 3A and CYP 2D6
- *Acetylation* is similar to that of man (dog is not)
- *Glucuronidation* is the predominant Phase II conjugation reaction
- Significant *glutathione-S-transferase* activity

Plasma elimination $t^1/_2$ (min)

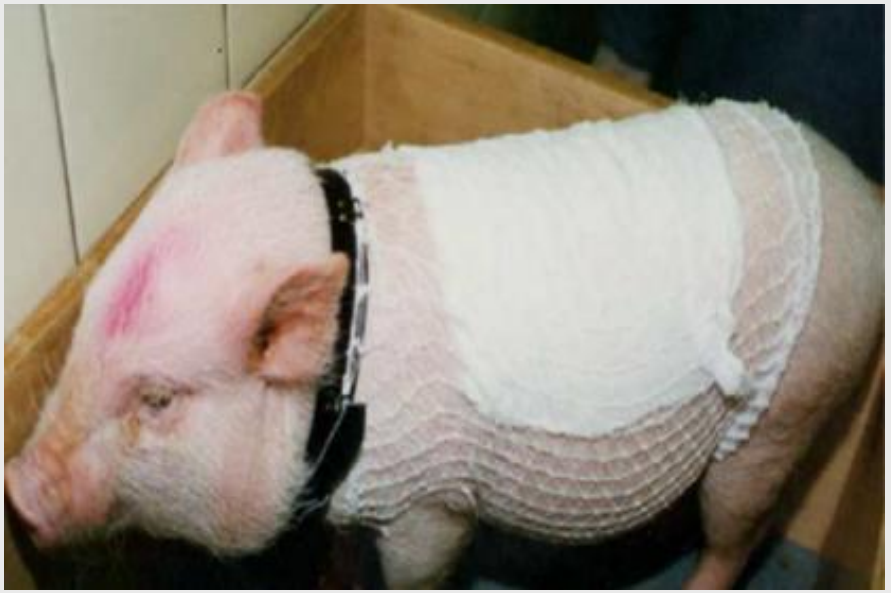
Drug	Minipig	Dog	Human	References
Acetaminophen	62	107	120	Bailie et al. (1987)
Vancomycin	88	102	330	Bailie et al. (1987)
Antipyrine	63	78	726	Bailie et al. (1987)
Cefepime	876	65	1,080	Elkhaili et al. (1997)
				Gardner et al. (2001)
Cefpirome	774		1,070	Elkhaili et al. (1997)
Meropenem	53	45	50	Elkhaili et al. (1997)
				Harrison et al. (1989)
Meloxicam	6,270	144	84	Busch et al. (1998)
Moxifloxacin (po)	660	540	720	Siefert et al. (1999)
Moxifloxacin (iv)	342	514	780	Siefert et al. (1999)

Minipig - Dermal administration

Courtesy of A. Mahl, Novartis Pharma AG



Open application



(Semi-)occlusive application

Similarities of porcine and human skin – 1

General morphology e.g.

- *Sparsely-haired*
- Dermis of *similar thickness* with similar vascular and elastic fiber network
- Well defined *papillary and reticular* dermal zones
- *Melanocytes* (pigmentation)
  Langerhans cells (immunology)
- Rete ridge structure, *firmly attached* to underlying structures

Similarities of porcine and human skin – 2

Physiology, e.g.

- *Cell turnover*
- Phase 1 and phase 2 *metabolic* activity
- *Immunological* reactivity
- Sensitivity to *ultraviolet* radiation
- Skin *penetration* of compounds
- Skin *repair* (wound healing)

Differences between porcine and human skin

- *Ichthyoform skin* surface, similar to thickened hyperkeratotic stratum corneum
- *Poor vascularization* of the cutaneous glands
- *Absence of eccrine and apocrine* glands
- *Seasonal* shedding of hair
- Differences in *skin microenvironment* (pH 6-7 compared to 5 in man)

Two valuable publications

- *The utility of the minipig as an animal model in regulatory toxicology*
  Bode G, Clausing P, Gervais F, Loegsted J, Luft J, Nogues V, Sims J
  J Pharmacol Toxicol Methods 2010 62(3):196-220
- *The RETHINK project on minipigs in the toxicity testing of new medicines and chemicals: conclusions and recommendations*
  Forster R, Bode G, Ellegaard L, van der Laan JW
  J Pharmacol Toxicol Methods 2010. 62(3): 236-42

Biology	**Macaque monkey**	***Human***
Weight	1.7-8 kg	*60-110 kg*
Life span	20-30 years	*60-90 years*
O_2 consumption	0.6 ml/g/hr	*0.4 ml/g/hr*
Heart rate	220-320/min	*60-90/min*
BP	120/70 mmHg	*120/80 mmHg*
Puberty	2-3 (f), 4-5 (m) years	*12-16 years*
Gestation	160-180 days	*40 weeks*
Weaning	12-27 months	*> 6 months*
Estrus cycle	28 days	*28 days*

“Monkeys” – Classification

- Old World (OW) monkeys (132 species)
  - E.g. macaque (cynomolgus / rhesus monkey), and baboon
  - *Characteristics:* narrow nose with nostrils facing downwards, trichromatic vision, shorter tail, eight premolar teeth (humans: 10), thumb opposable (like man)
- New World (NW) monkeys (135 species)
  - E.g. marmosets and squirrel monkey
  - *Characteristics:* flat nose with side-facing nostrils, dichromatic vision, prehensile tails, twelve premolar teeth, mostly monogamous, thumb not opposable
- Great apes
  - Lesser apes: gibbon
    Greater apes : chimpanzees, gorilla, orangutan
  - *Characteristics:* no tail

“Monkeys” – Use (first decade of 2000)

- Genotypic and phenotypic resemblance to humans, especially regarding
  - Immune system
  - Kidney
  - CV system
  - Etc.
- Numbers used/year:
  - EU (~ 500 mio inhabitants) ~ 10’000
  - UK (~ 62 mio inhabitants) ~ 4’000
  - USA (~ 310 mio inhabitants) ~ 55’000

Non-human primates (NHP) – 1

- *Expensive, limited supply, potential quality issues*
- *Handling* not that easy
  Special requirements for husbandry including environmental enrichment
- *Animal protection issues* in certain parts of the world
- *Infectious diseases* may be transmitted to humans

Non-human primates (NHP) – 2

- *Smaller than dogs* ➔ less substance required (3 kg macaque vs. 10 kg dog)
- *Sensitive* to thalidomide and other drugs
- Biotechnology products: *pharmacological responsiveness*
- *Placenta* similar to humans (discoidal, hemomonochorial) in contrast to other species
- *Sperm production* (#/g testis/d) similar to rat and dog and approx. 7 times higher than humans

Advantages of marmosets

- *Breed easier* → Local supply and less transport stress (Japan?)
- Easier to obtain *sexually mature* animals
- Easier to *handle*
- On a *lower phylogenetic plane*, but higher than dogs (positive and negative!)
  Pain sensation probably similar to dogs
- Generally significantly *smaller:* a few hundred gr vs. a few kg (macaque)

Advantages of macaques

Sometimes

- Better express certain *receptors*
- *Lack immune response* against some compound

Most frequently used NHP: ~ 80 % in UK, > 60% of federally funded US research

→ More *experience* and more drugs tested of testing

Homology of CYP P450 enzyme

Species	*P4503A homology with human sequence)*
Human	100
Cynomolgus macaque	93
Marmoset	90
Dog	79
Minipig	74
Rat	73

Examples of drugs tested with NHP

- Testing for myopathy with HMG CoA reductase inhibitors
- PPAR tumor induction (rodents)
- Biotechnology products
- ACE inhibitor
- Proteinase inhibitors
- *Parkinsonism:* MPTP (neurotoxin) model

However, monkeys do not always predict – 1

- *Clinical failure of AIDSVAX* after successful results in chimpanzees
- Prediction of *isoprenaline doses for asthma* with NHP not better (Carson et al, 1971)
- *Adverse drug reactions to hormone replacement therapy*
  - Based on NHP experiments thought to protect against heart disease and stroke
  - Now known to *increase the risk of these diseases and lead to breast cancer*
- *Amrinone* (for heart failure) caused *frequently severe and hemorrhages* not predicted by NHP testing (Eason et al, 1987)

However, monkeys do not always predict – 2

- *Teratology:* NHP results correlate only in 50%
  Less than rats, hamsters and ferrets (Schardein, 1993; Bailey et al., 2005)
  Example: *Aspirin is teratogenic in monkeys* but not in humans
- Opren and Flosint *(antiarthritis drugs)* toxic for humans but without problems in NHPs (Eason et al, 1990) (Mann, 1984)
- *Carbenoxalone* (for gastric ulcers) associated heart failure not reproducible in NHPs (Eason et al, 1990)

Alternatives to NHP

- Dogs
- Ferret, but e.g.
  - Not much background data
  - Regulatory acceptance
  - Supply issues
- Minipig, but
  - Higher test compound requirements


Curteousy of Andy Meier, Biel-Benken

Overview

- Use of animals
- Regulations regarding species selection
- Species similarities and differences
- Commonly used species in toxicology
- Humans
  - Ethnic variability
  - Individual variability
- Adverse drug reactions in humans
- Extrapolation of animal data
- Species selection
- Conclusions

E5(R1) Ethnic Factors in the Acceptability of Foreign Clinical Data

Main points

- Populations classified as Asians, Blacks and Caucasians
- Most drugs are ethnically not sensitive
- Assessment based on clinical bridging data *not mandatory* in every case, but *may be required*
- List of factors, which make it less likely that drug in question is sensitive to ethnic factors: *see next slide*

Factors indicating potential ethnic sensitivity

- *Nonlinear* pharmacokinetics
- *Steep* dose-response curve (pharmacology and toxicology)
- *Narrow* therapeutic window
- *Strong* metabolism, especially through a single pathway, thus increasing the potential for drug-drug interactions
- Metabolism by enzymes with *known polymorphism (see later)*

Further factors for potential ethnic sensitivity

- *Pro-drugs*
- High *variability* in bioavailability
- *Low bioavailability*, thus increased susceptibility to dietary influences
- Therapeutic indication with likely *co-medication*
- Potential for *abuse*

Examples of genetic variations influencing drug effects

Mechanism	Example	Pharmacogenetic effect
Drug metabolism	Cytochrome P450 *enzymes*, e.g. CYP2D6	"Poor metabolizer" of importance for 25% of all drugs *(see next slide)*
Drug target	Serotonin (5-HT2A) *receptor*	Altered binding of the atypical antipsychotic clozapine
Disease pathway	Cholesterol esterase *transport protein* (CETP)	Atherosclerosis progression and response to the HMG-CoA inhibitor pravastatin

Kleyn PW, Vesell ES. Genetic variation as a guide to drug development Science.1998; 281:1820–1821

Frequency of poor acetylators

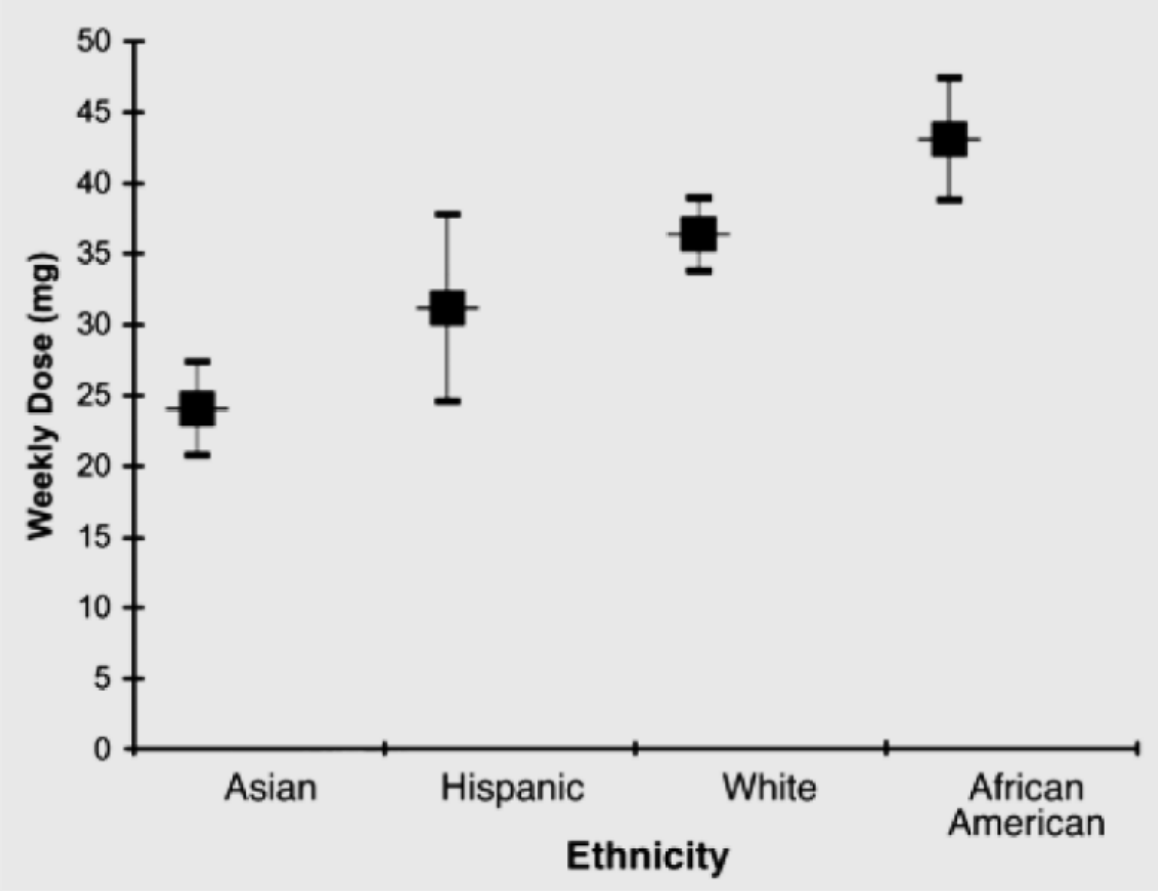
Population	Median	Range
Black	51	42-65
Caucasian	58	52-62
Chinese	22	13-34
Japanese	10	7-12
Eskimo	6	5-21

Wood AJ, Zhou HH.
Ethnic differences in drug disposition and responsiveness
Clin Pharmacokinet 1991;20:350 –373

Ethnic polymorphism of hepatic metabolism

Enzyme		**Metabolized drug**
CYP2C9		S-warfarin *(see next slide)*
CYP2D6		Neuroleptics, tricyclic antidepressants, doxorubicin, codeine, propranolol, haloperidol
CYP2A2		Theophylline, imipramine, clozapine, olanzapine
N-acetyl-transferase	NAT-2	Isoniazid, hydralazid
	NAT-1	p-Aminosalicyclic acid

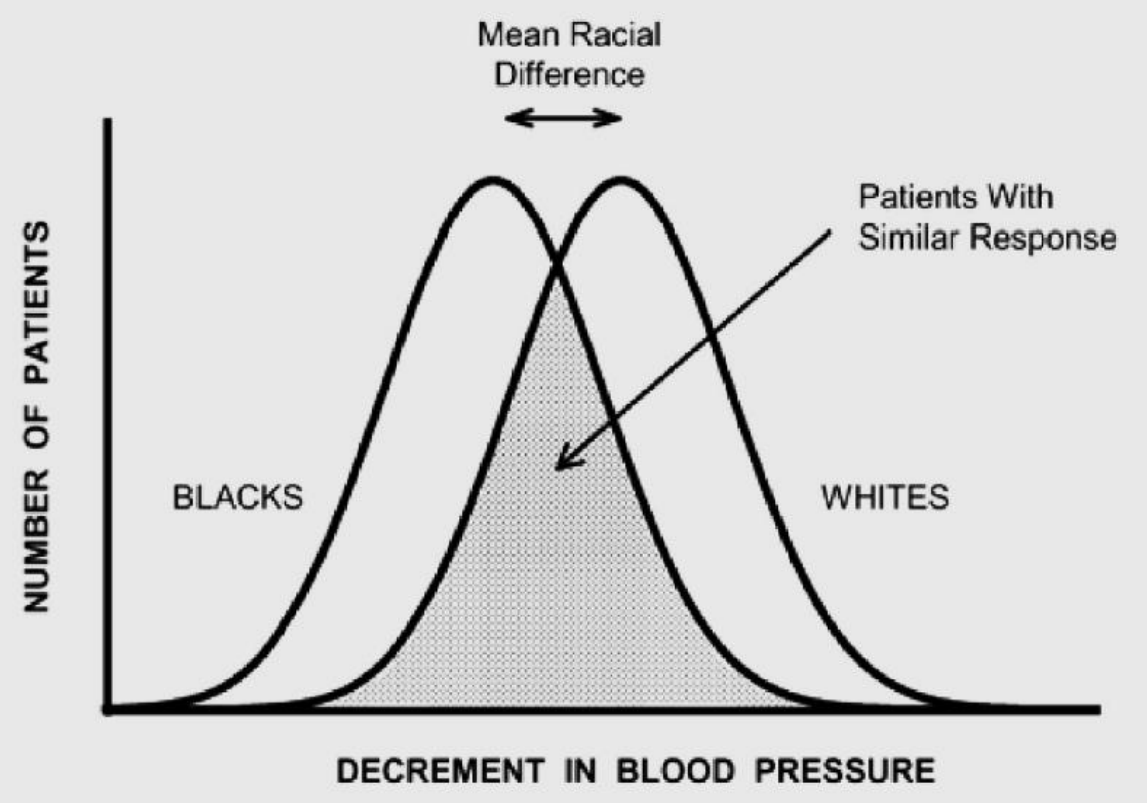
Warfarin requirements



Average warfarin dose required to maintain a thera-peutic international norm ratio of 2-3
(formerly Quick value)

Dang MT, Hambleton J, Kayser SR
The influence of ethnicity on warfarin dosage requirement
Ann Pharmacother 2005 39(6): 1008–1012

Response to antihypertensives



Decrement in blood pressure with antihypertensive drug such as ACE inhibitors, ARBs or β-blockers.

Sehgal AR

Overlap between whites and blacks in response to antihypertensive drugs

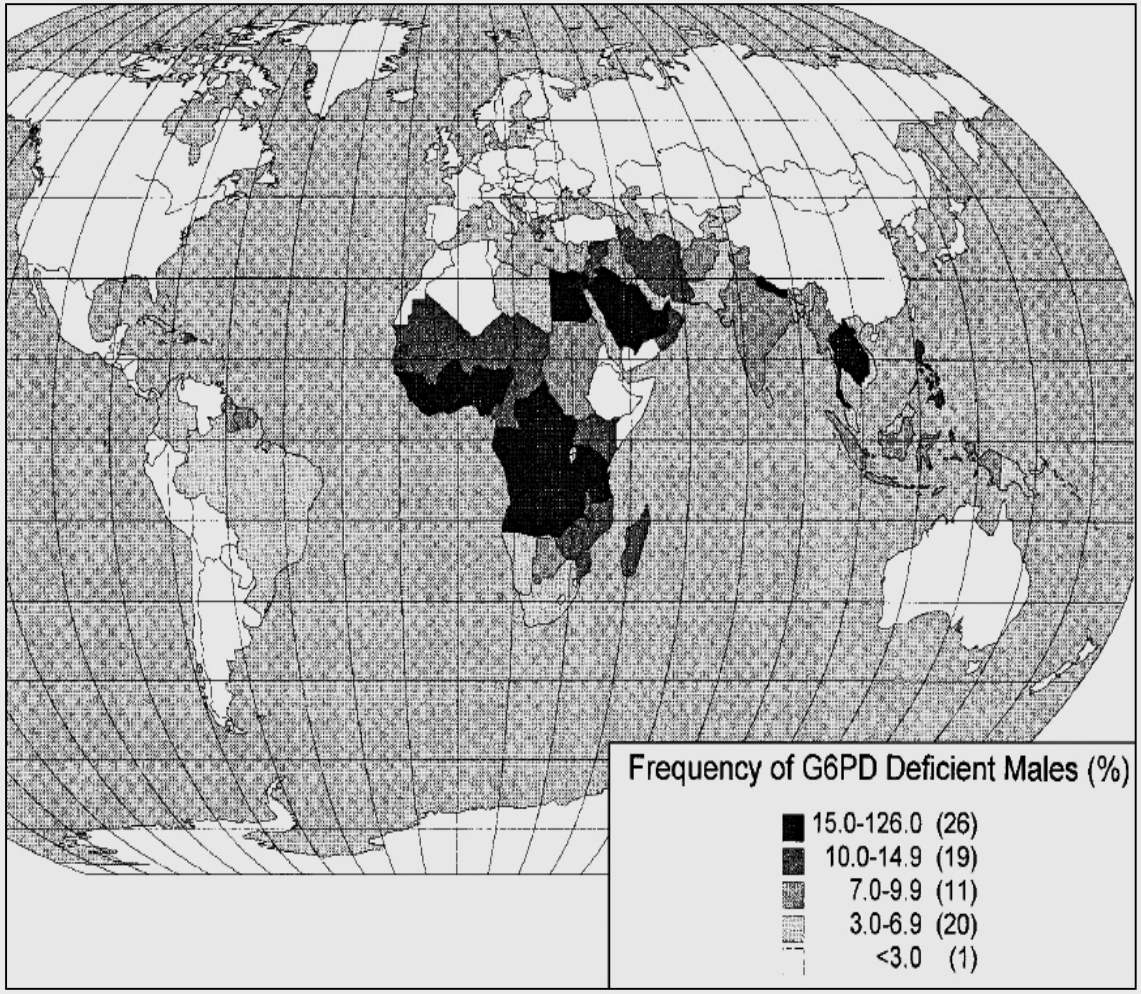
Hypertension 2004 43(3): 566–572

G6PD-deficiency: favism

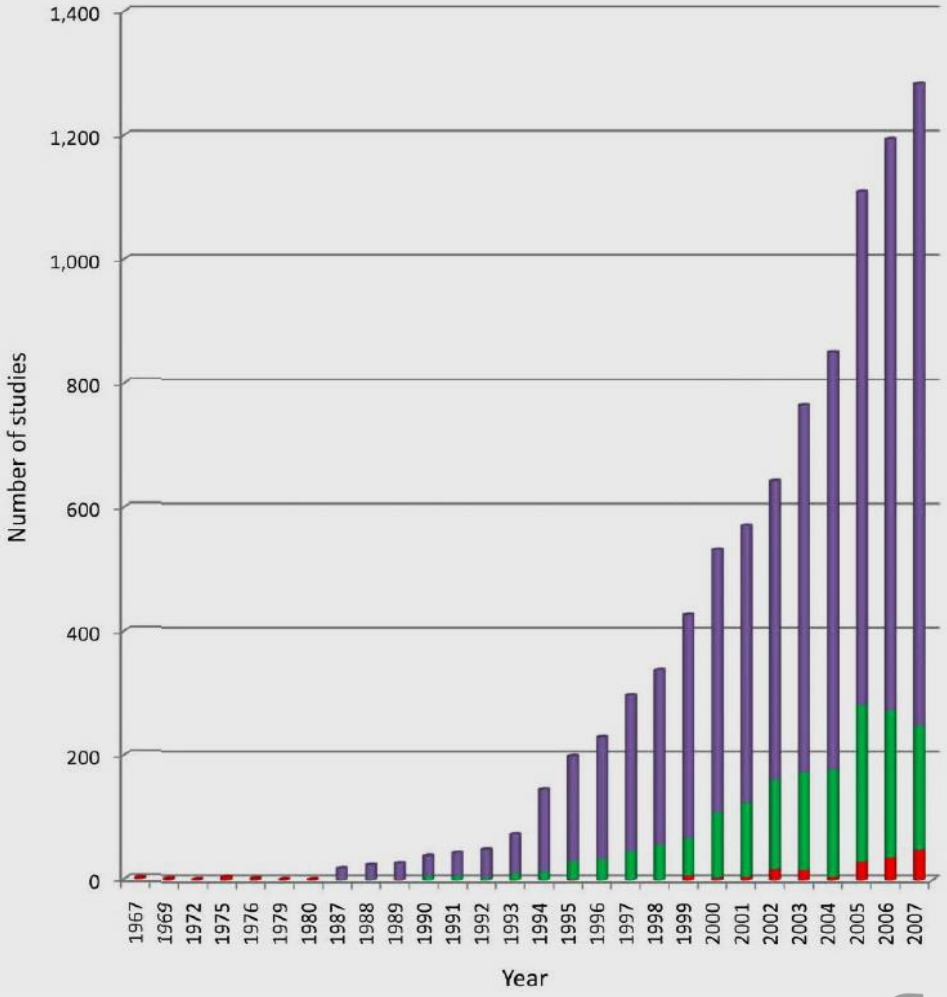
Many drugs are contraindicated, among them certain

- Analgesics
- Antimalarial
- CV drugs
- Sulfonamides
- Antibiotics
- Anticancer drugs
- etc.

Was available under
http://www.rialto.com/g6pd/table2.htm



publications about pharmacogenetics 1967–2007



Broad search with Medical Subject Headings and free-text terms: 6,548 original articles

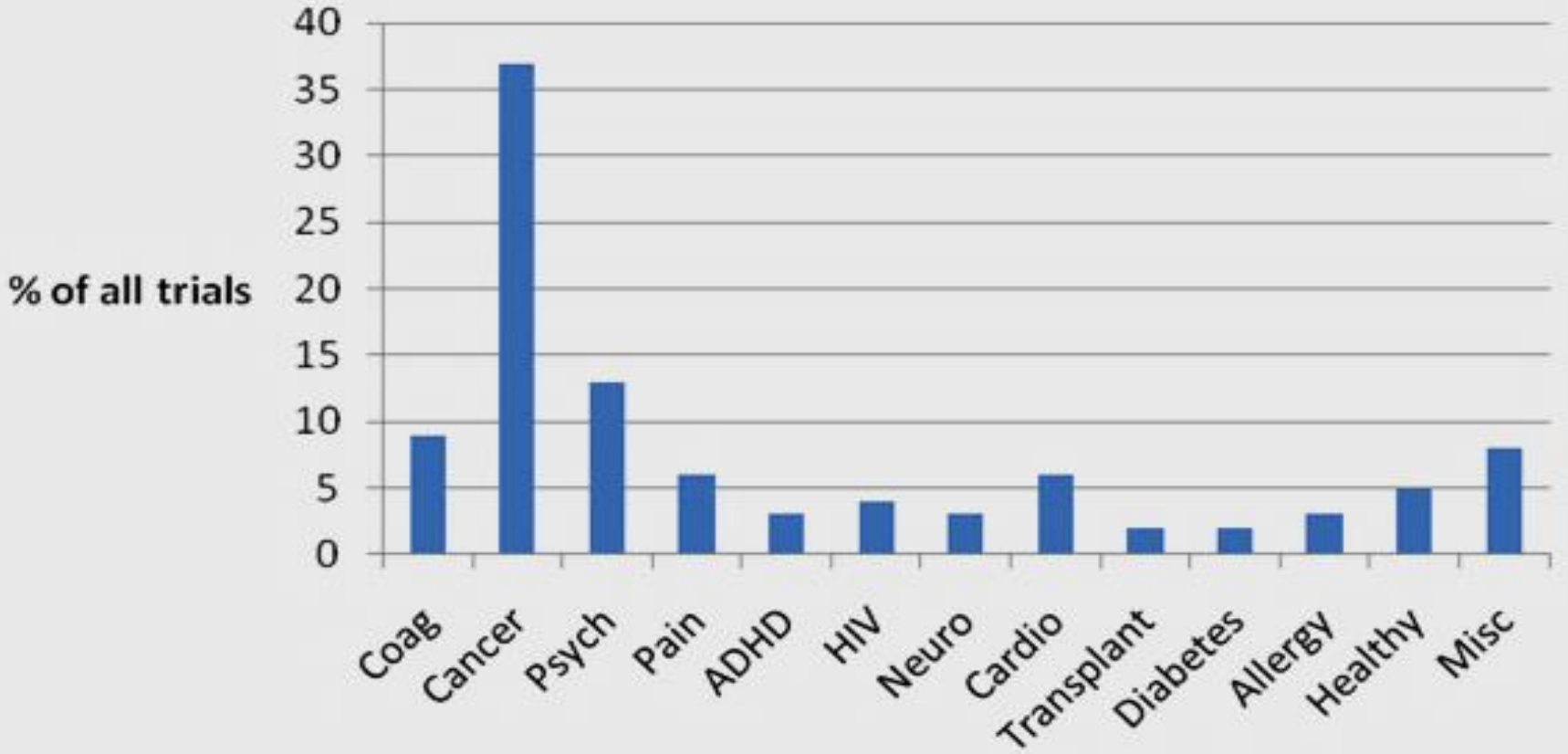
1,668 primarily PG-related

183 were original PG articles

→ Ratio of approximately 1(original research) : 25 (commentary/review)

PLoS One. 2009, 4(12): e7960 - Holmes MV et al.

Pharmacogenetic trials



Percentage of pharmacogenetic trials (n=158) registered as of April 2011 with clinicaltrials.gov by disease process or condition

Carlquist JF and Anderson JL. Discov Med 2011,11(60): 469-78

Individual vs. population response

- *Variability* of the response to a drug is likely to be similar, if not greater, *between individuals of the same population*, when compared to that between different populations
- Countries, which ask for proof of absence of ethnical differences more often than others: *Japan, (South) Korea, Taiwan*

Stratified (individualized) medicine

- Practiced since many years
  - Pharmacogenetic investigations
  - Consideration of non-genetic factors including e.g.
    - Age
    - Comorbidities
    - Concomitant medications
    - etc.

➔ Decision

- *Which drug* to prescribe
- *Dose adjustments*

Overview

- Use of animals
- Regulations regarding species selection
- Species similarities and differences
- Commonly used species in toxicology
- Humans
- Adverse drug reactions in humans
  - General
  - Interactions
  - Risk factors
- Extrapolation of animal data
- Species selection
- Conclusions

Frequency and grading of ADRs

In/out-patients	ADR grade	# of studies	Total patients studied	Incidence of ADRs %	95% confidence limits
Admission due to ADR	Serious	21	28 017	4.7	3.1-6.2
	Fatal	6	17 753	0.13	0.04-0.21
ADRs in hospital	Total	18	34 463	10.9	7.9-13.9
	Serious	12	22 502	2.1	1.9-2.3
	Fatal	10	28 872	0.19	0.13-0.26

Lazarou et al. JAMA. 1998;279:1200-1205

Costs associated with ADRs in the USA

- Well above *100 bio US $* per year
- Patients with ADR: on average *doubling* of
  - Mean length of stay
  - Cost
  - Mortality
- 69% of *fatal ADRs* are caused by
  - Anticancer drugs
  - Cardiovascular drugs
  - CNS drugs

Why development safety sometimes fails?

- Most drugs approved with data of an average of *1500 patients*
- Some drugs have *rare* toxicity profiles (bromfenac hepatotoxicity 1 in 20,000 patients)

  Data of > 100,000 patients needed to generate a signal

  → Importance of post-marketing surveillance

ADRs *would* often be preventable – 1

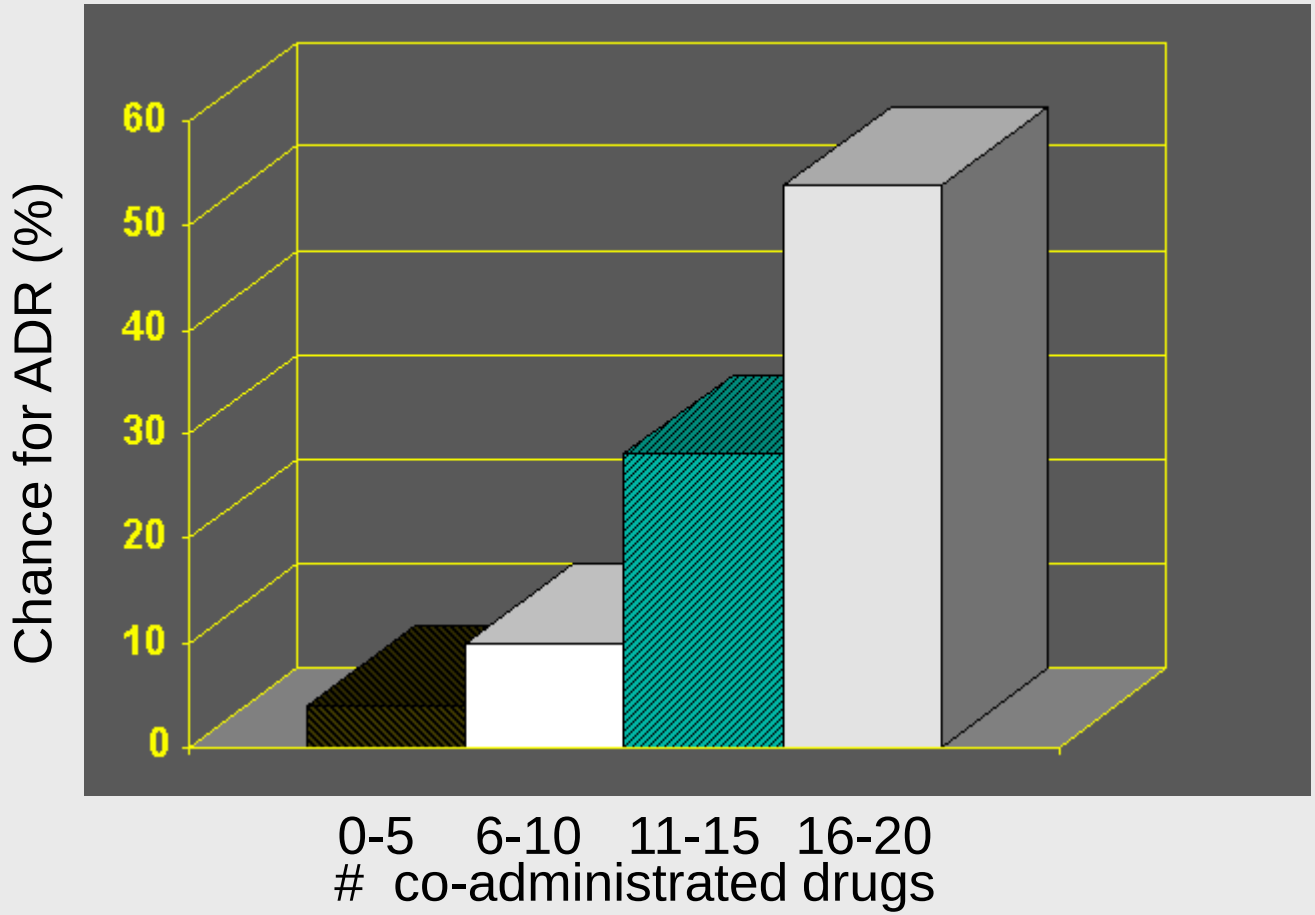
Prescription habits in the USA

- Two-thirds of patient visits result in a prescription
- 2.8 bio outpatient prescriptions (10 per US citizen!) in 2000
- ADRs increase exponentially with 4 or more medications

Schappert SM. Vital Health Stat. 1999;13(143).
2000 community pharmacy results. 2001
Jacubeit T et al. Agents Actions 1990; 29:117–125

ADR as f (number of drugs)

May FE. Clin Pharmacol Ther 1977; 22:322-328



ADRs *would* often be preventable – 2

> 80% of ADRs causing admission or occurring in hospital are dose-related (type A ARDs)

➔ Predictable (potentially) avoidable

Adverse drug reactions in elderly patients - P A Routledge, M S O'Mahony, K W Woodhous
Br J Clin Pharmacol 2004, 57(2): 121–126

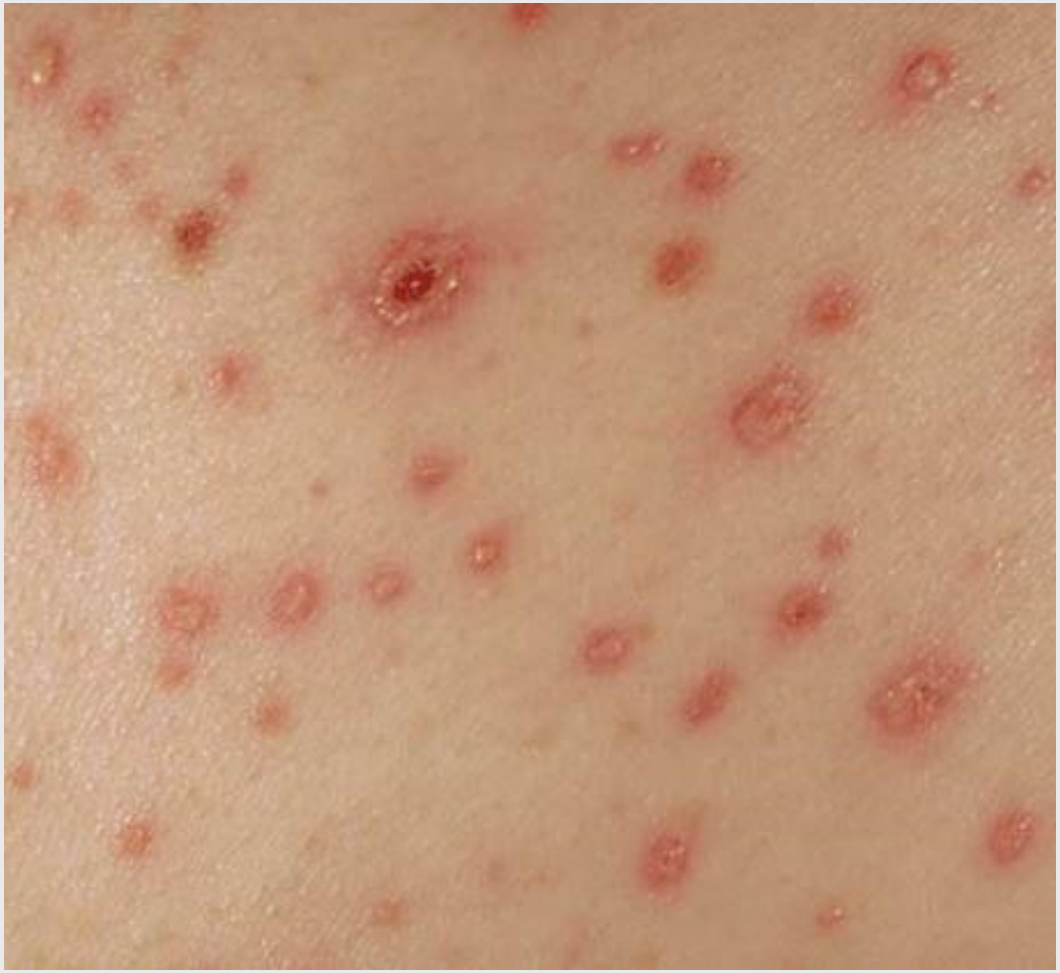
Mechanisms of “idiosyncratic” (Typ B) ADR

- *Receptor abnormality:* malignant hyperthermia with general anesthetics
- *Biological variation*
  - Primaquine induced hemolysis in patients deficient in *glucose 6-phosphate dehydrogenase*
  - Isoniazid-induced peripheral neuropathy in people *deficient in N-acetyl transferase* (“slow acetylators”)
- *Certain types of drug-drug interactions:* increased incidence of hepatitis of isoniazid in combination with rifampicin
- *Multifactorial pathogenesis:* halothane hepatitis
- Pharmaceutical “variation”: eosinophilia-myalgia syndrome with *“impurities”* in L-tryptophan (New Mexico 1989)
- *Immunological reaction:* see next slide

Immunologic ARD (idiosyncratic)

Type	Example
Type I reaction (IgE-mediated)	Anaphylaxis from lactam antibiotic
Type II reaction (cytotoxic)	Hemolytic anemia from penicillin
Type III reaction (immune complex)	Serum sickness from antithymocyte globulin
Type IV reaction (delayed, cell-mediated)	Contact dermatitis from topical antihistamine
Specific T-cell activation	Morbilliform rash from sulfonamides
Stevens-Johnson syndrome	Toxic epidermal necrolysis
Fas/Fas ligand-induced apoptosis	Drug-induced lupus-like syndrome
Other	Anticonvulsant hypersensitivity

Most frequent: skin rash



- 90% due to drug hypersensitivity
- Mostly transient, resolving in 6-9 days
- Rarely severe – 1/10’000 patients
- Not predictable from conventional animal studies

Preventable Adverse Drug Reactions: A Focus on Drug Interactions - Learning
http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm110632.htm

Interactions

1. Interactions can occur already before administration
2. PK interactions
   a. GI tract
   b. Plasma
   c. Liver function
   d. Kidney function
3. PD interactions in the target organ

1. Interactions before administration

- Phenytoin precipitates in dextrose solutions (e.g. D5W)
- Amphotericin precipitates in saline
- Gentamicin is physically/chemically incompatible with most beta-lactams, resulting in loss of antibiotic effect

2a. Interaction in the GI Tract

Agent	Effect
Some milk products, *oral iron preparations,* antacids, sucralfate	*Block absorption* of quinolones, tetracycline, and azithromycin
Omeprazole, lanso-prazole, H2-antagonists	*Reduce absorption* of ketoconazole, delavirdine
Didanosine (given as a buffered tablet)	*Reduces absorption* of ketoconazole
Cholestyramine	*Binds* raloxifene, thyroid hormone, digoxin

Preventable Adverse Drug Reactions: A Focus on Drug Interactions - Learning
http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm110632.htm

2b. Interactions in the plasma

- Some drugs can displace other drugs off proteins in the plasma
  → Increased *amount of free drug*
- Generally only transient effect

  → Increased rate of elimination and normalization of blood levels of free drug

Preventable Adverse Drug Reactions: A Focus on Drug Interactions - Learning
http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm110632.htm

2c. Interactions due to hepatic metabolism

- Nearly always due to interactions involving *Phase I enzymes* (mainly P450), rather than Phase II
- *60%* of drugs frequently cited in connection with ADR are metabolized by *enzymes with known genetic variations*

Preventable Adverse Drug Reactions: A Focus on Drug Interactions - Learning
http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm110632.htm

2c. Drug-drug interactions affecting e.g. lipid-lowering drugs (competition for enzymes)

Primary drug (PD)	Concomitant drug	Increase in AUC of PD
Simvastatin	Itraconazol	5 - 20 x
Lovastatin	Itraconazol, cyclosporine	5 - 20 x
Atorvastatin	Cyclosporine	6 – 15 x
Fluvastatin	Cyclosporine	2 – 4 x
Cerivastatin	Gemfibrozil	4 - 6 x
Pravastatin	Cyclosporine	5 – 10 x
Rosuvastatin	Cyclosporine	5 – 10 x
Pitavastatin	Cyclosporine	5 x

Neuvonen PJ et al, Clin Pharmacol Ther 80(6): 565–581 (2006)

2d. Affected by decreased renal clearance

Drug class	Representative medications
ACE inhibitors	Donepezil, rivastigmine
Analgesics	Acetaminophen, NSAIDs, morphine, fentanyl
Antibiotics	Aminoglycosides, fluroquinolones, carbapenem, b-lactams, cephalosporins, penicillins, sulphonamides, nitrofurantoin
Antivirals	Amantidine, oseltamivir, famicyclovir, acyclovir
CV drugs	Beta blockers, diuretics, digoxin, procainamide
Diabetic drugs	Metformin, chlorpropamide, tolazamide, glyburide
Other drugs	H2 antagonists, lithium, antipsychotics, venlafaxine, allopurinol, gabapentin

Was available under http://www.fmpe.org/en/documents/doc_aids/aid_kidney_disease_apd4.pdf

3. Pharmacodynamic (PD) interactions

E.g.

- *Overlapping (additive)* toxicities, e.g. ethanol and benzodiazepines
- *Antagonistic* effects, e.g. anticholinergic medications such as amitriptyline (monoamine re-uptake inhibitor) and acetylcholinesterase inhibitor

Deliberate interactions:

- Synergistic actions of antibiotics: penicillins – gentamicin
- Parkinson therapy: levodopa - carbidopa
- Hypertension treatment: ACE inhibitors – thiazides
- Asthma bronchiale therapy: salbutamol - ipratropium

Drug - food or herbal interactions – 1

- *Calcium in milk products* chelates tetracyclines
- *Vitamin K-containing foods* antagonizes anticoagulation by Warfarin
- *Grapefruit juice* contains a bioflavonoid that inhibits CYP3A and blocks the metabolism of many drugs

Preventable Adverse Drug Reactions: A Focus on Drug Interactions - Learning
http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm110632.htm
Accessed in January 2021

Drug - food or herbal interactions – 2

St. John's wort (hypericum perforatum), used e.g. as antidepressant, interacts with

- Indinavir
- Cyclosporin
- Digoxin
- Other drugs

by induction of CYT P450 and/or drug transporters

Preventable Adverse Drug Reactions: A Focus on Drug Interactions – Learning-
http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm110632.htm (accessed 01/21)

Risk factors for ADR - General

- Age (children and elderly)
- Gender (females)
- Environment, diet, drinking habits, etc.
- Multiple medications, medical practice
- Multiple co-morbid conditions including end-organ dysfunction
- Prior history of ADRs
- Dose and duration of exposure
- Genetic predisposition

Modifying factors

- Personal susceptibility

  Exaggerated response, e.g. 10 mg of morphine > up to 10 – 12 hrs. of sleep (generally only 4 – 6 hrs.)

- Effect of climate
  - In hot humid climate metabolism is depressed
  - Purgatives act better during summer
  - Diuretics act better during winter
- Effect of altitude

  Increasing height reduces metabolism
- Racial Differences
  - Castor oil: not purgative to Chinese
  - Ephedrine: no dilatation of pupil in black people

Learning module on DDI

FDA

Preventable Adverse Drug Reactions: A Focus on Drug Interactions

http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm110632.htm
(accessed in January 2021)

Overview

- Use of animals
- Regulations regarding species selection
- Species similarities and differences
- Commonly used species in toxicology
- Humans
- Adverse drug reactions in humans
- **Extrapolation of animal data including risk assessment**
- Species selection
- Conclusions

For risk assessment

Review: Successful Drug Development Despite Adverse Preclinical Findings

Part 1: Processes to Address Issues and Most Important Findings

Robert A. Ettlin, Junji Kuroda, Stephanie Plassmann, and David E. Prentice

J Toxicol Pathol 2010; ***23****: 189–211*

Part 2: Examples

Robert A. Ettlin, Junji Kuroda, Stephanie Plassmann, Makoto Hayashi, and David E. Prentice

J Toxicol Pathol 2010; ***23****: 213–234*

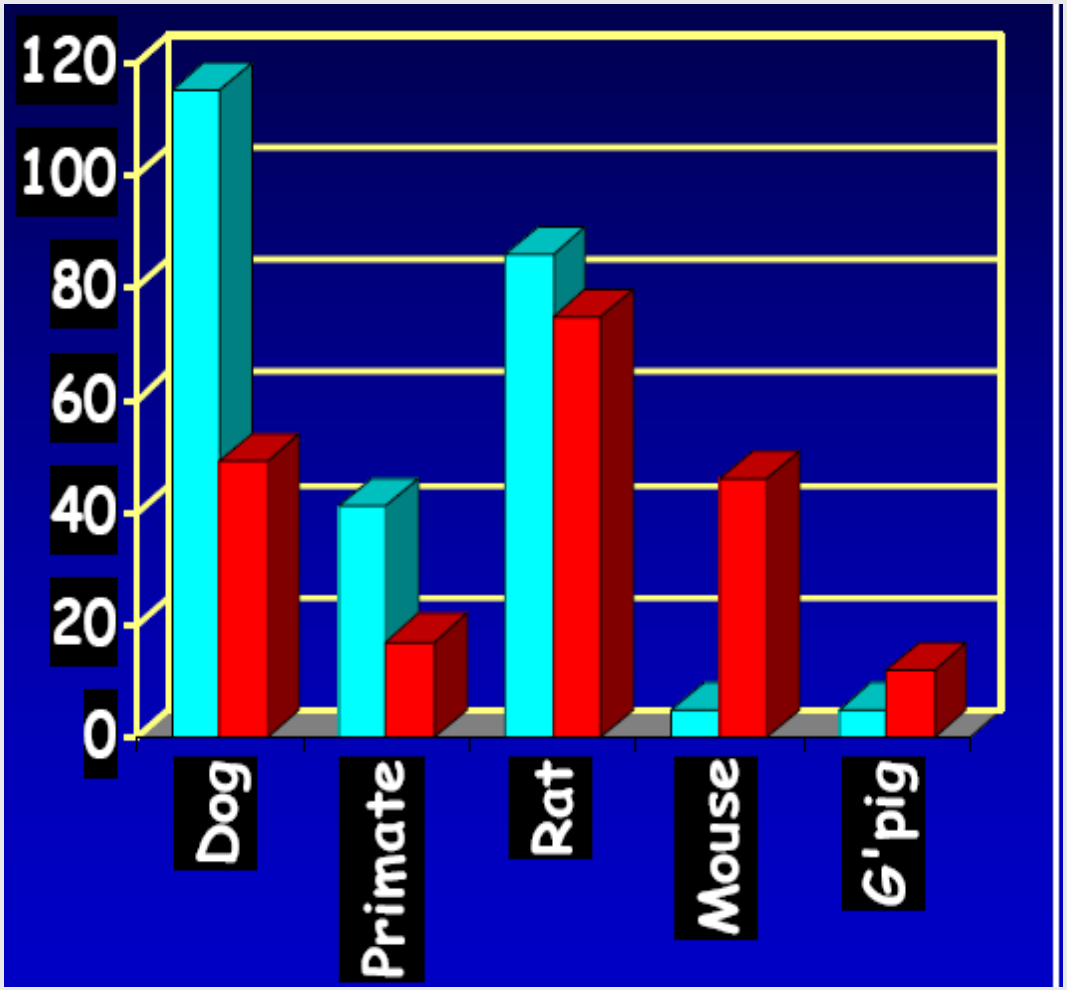
http://www.jstage.jst.go.jp/browse/tox/23/4/_contents

Concordance of findings with drugs in humans and animals – 1

- 150 compounds
- 12 companies
- Analyzed by ILSI

Olson H et al. Regul Toxicol Pharmacol 32, 56-67, 2000

Concordance of findings with drugs in humans and animals – 2



Overall concordance: 71%

- Non-rodents alone 63%
- Rodents alone 43%

Differences between tissues

Missing concordance is not explained by differences in metabolism

Regul Toxicol Pharmacol. 2000 Aug;32(1):56-67.
Concordance of the toxicity of pharmaceuticals in humans and in animals.
Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken W, Dorato M, Van Deun K, Smith P, Berger B, Heller A.

Extrapolation between species

In view of the fact that human beings react partly very differently to drugs:

Differences between individual human beings

are probably in a similar order of magnitude

as the differences between test animal species and humans

Overview

- Use of animals
- Regulations regarding species selection
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- Commonly used species in toxicology
- Humans
- Adverse drug reactions in humans
- Extrapolation of animal data
- **Species selection**
  - General
  - Non-human primates
  - Examples: dermal and inhalation studies
- Conclusions

Animals can serve as predictive models

- Mechanisms of chemical toxicity are largely identical in humans and animals
- Agents found toxic in animals are generally not knowingly given to humans

  → *Biased data samples* to investigate the effectiveness of animal testing

Curteousy of Andy Meier, Biel-Benken

Selection of animal species

- The selection of an appropriate species is *key to accurate prediction* of safety in humans.
  The theoretical basis is available, but possibly more often quoted than adhered to
- It is virtually *impossible to give specific rules*
- Considerations *depend on the specific research project and its objectives*
- To select a species means to *understand and weigh advantages and disadvantages* of the various species (an issue of trade-offs)

SELECTION OF ANIMAL MODELS Research Animal Methods - VSC 443/543 - Fall 2009
Michael S. Rand, DVM, Assistant Director, University Animal Care - University of Arizona – Tucson - Lecture date: 9/18/2009
Was available under http://www.uac.arizona.edu/VSC443/animalmodels/animalmodels09.html

Factors to be considered – 1

- What needs to be shown?
  For “screening” wider range of appropriate species
- Similarity to humans regarding
  - Anatomy and physiology
  - Age equivalence
  - PD and TK (ADME); influences also compound requirements
  - PD response and tissue binding
  - Etc.

Factors to be considered – 2

- Economics of the species selection
  - *Purchasing* costs
  - *Husbandry* costs, influenced also by space / regulatory requirements
  - *Compound* requirement based on
    - Body size
    - Test group size
    - ADME considerations
    - Etc.

Factors to be considered – 3

- Practical aspects
  - Availability of species
  - Requirements for husbandry
  - Life span
  - Ease of handling
  - Ability to make pertinent measurements in a meaningful way, e.g. size / numbers of samples, availability of methods, etc.
  - Spontaneous diseases including those transmissible to humans

Factors to be considered – 4

- Experience
  - Historical data
  - Handling experience
  - Experience with animal model
  - Data on compounds of the same chemical and therapeutic class
- Ethical considerations
- Regulatory acceptance
- (Habit, personal preferences)

Data for non-rodent species selection – 1

Ideally

- Exploratory *toxicity* data from the rodents *(e.g. rats)*
- *PD* activity in non-rodent species
- *Cardiovascular* telemetry data (including electrocardiogram ECG) of different species after low single doses
- Information from *precursor* compounds (if applicable)

After G Bode et al. J Pharmacol Toxicol Methods 2010, 62: 196-220

Data for non-rodent species selection – 2

- Some ADME information, including e.g.
  - *In vivo p.o./i.v. plasma profiles* of active compound in rats, dogs and/or monkeys and minipigs
  - *In vitro metabolism* in liver microsomes and/or hepatocytes of rats, dogs, minipigs, non-human primates and men
  - Early assessment of metabolite(s) if identified as *major contributors to drug/toxicity action*

After G Bode et al. J Pharmacol Toxicol Methods 2010, 62: 196-220

Reason for choosing NHP

- With conventional studies *progression in clinics* was questionable *(see next slide)*
- Testing in monkeys has allowed to *terminate development* before exposing humans *(see next slide)*
- *Scientific* reasons, e.g.
  - No antigenicity of test drug
  - Similarity of receptors to those of humans
- *Experience* with same class of compounds in NHP
- *Economical reasons:* amount of compound needed
- *Caution:* avoid risk that choosing another species may later prove unacceptable to the regulators resulting in costly delays
- To *"please"* the regulator

After 'Use of non-human primates in regulatory toxicology - Was available under http://www.boyd-group.demon.co.uk/

Examples of benefits of NHP

- Anti-schizophrenic drug produced *cataracts* in dogs. NHP without eye toxicity
  Clinical use showed better efficacy and tolerance than previous treatment options (without eye ADR)
- Safety evaluation of a cholesterol-lowering compound in marmosets showed *muscle damage* → compound dropped
  The marmoset was chosen for its lipoprotein profile being similar to that of humans

However, monkeys are not always better than other non-rodents or rodents *(see earlier)*!

Modified after Use of non-human primates in regulatory toxicology – Was available under http://www.boyd-group.demon.co.uk/

Decision tree non-rodent species

Species	Limitations	Comments
(Ferret)	Availability Experience	Currently no option
Minipig	Size (Background data)	To avoid primates
Dog	Scientific suitability Size (occasionally)	Primary species
Marmoset	Sampling Monitoring	On lower phylogenetic plane than macaque
Macaque	Last option	Up to 10 times heavier than marmosets

According to 86/609 EEC Revision Nov. 5, 2008 “Protection of animals”

Examples for species selection

Dermal studies

Comparison of anatomical characteristics of the skin for several laboratory animals
John H Grabau , Lily Dong, 1995

Was available under http://www.dtic.mil/cgi-bin/GetTRDoc?AD=ADA325862&Location=U2&doc=GetTRDoc.pdf

Inhalation studies

The relevance of animal models for aerosol studies
Phalen RF, Oldham MJ, Wolff RK
J Aerosol Med Pulm Drug Deliv 2008, 21(1): 113-24

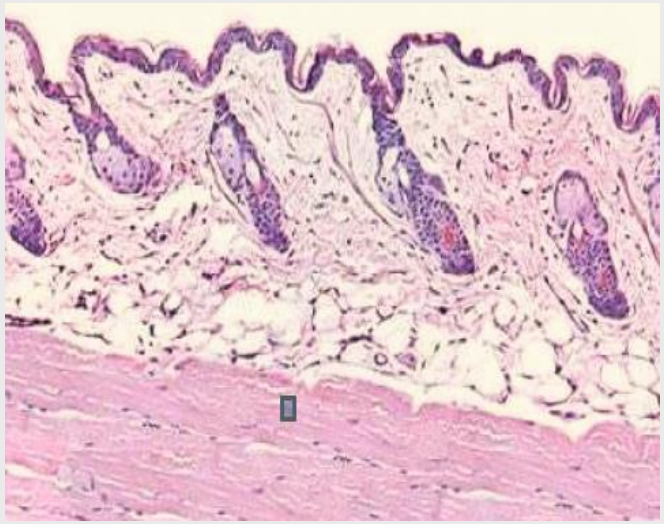
Dermal studies

In vitro skin permeability to ionic and covalent substances in aqueous solution and organic solutes

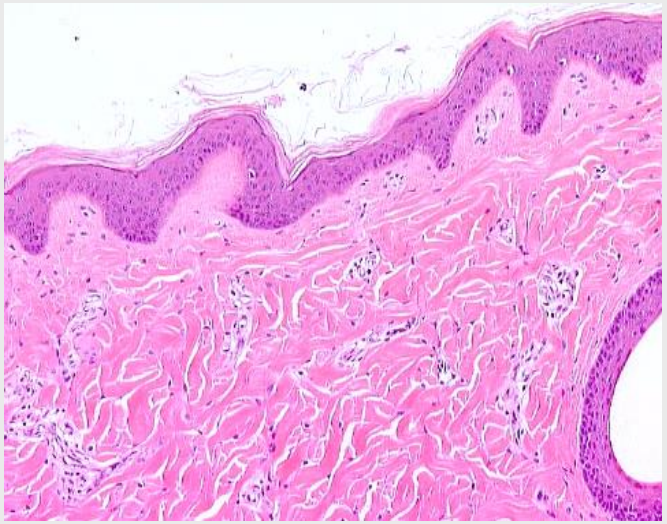
Human < pig < guinea pig < rat < rabbit

Physical functions of skin. R. T. Tregear. Academic Press, London, New York, 1966

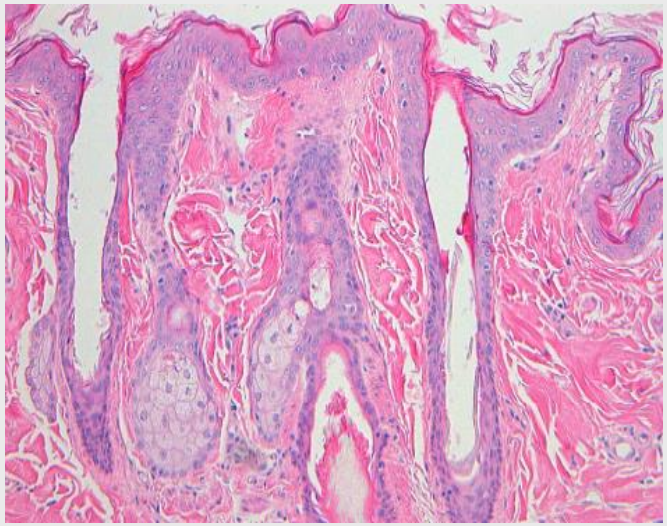
Skin including epidermis thickness



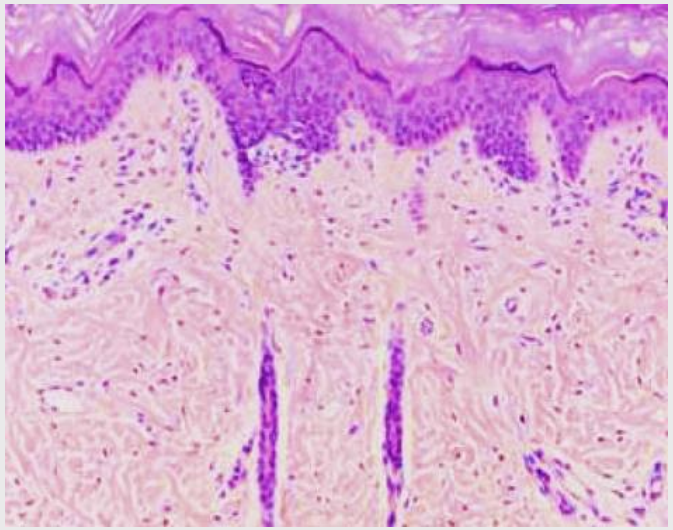
Mouse – 13 µm (rat 21.7 µm)



Pig – 52 µm



Monkey – 27 µm



Human – 70 µm

Courtesy of A. Mahl, Novartis Pharma AG

Inhalation studies

- *Mouth* (humans, 10µ filtration) versus *nose* (rodents, 3µ filtration) breathers
- *Anatomy*
  - Number of branchings in the respiratory tree (humans: 35, rodents:?)
  - Respiratory bronchioles / submucosal glands in intrapulmonary bronchi:
    - Extensive / present in dogs, rhesus and humans
    - Minimal / absent in rodents and hamsters

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  With a look into the future

Animal models

- Have proven their *value* to (generally) protect human beings from serious adverse drug effects
- Significant *progress* was made in understanding the strengths *(similarities)* and weaknesses *(differences)* of animal testing for predicting adverse effects in humans

Animal species used in regulatory toxicology

Study type	Primary species	Alternatives
Acute toxicity	Rodents (rat, mouse)	
Exploratory safety testing	Rat, dog	Monkey, minipig
Multidose toxicity	Rodent (especially rat) Non-rodent (especially dog)	Monkey, minipig
Carcinogenicity	Rodents: rat, mouse(**?**)	
In vivo mutagenicity	Mouse	Rat Non-rodent species?
Development / reproductive tox	Rat, rabbit	(Mouse, hamster, monkey)
Immunotoxicity	Mouse, guinea pig	Rat

A look into the future – 1

- We will continue using *animals,* but we will have to continue increasing our understanding of their limitations in connection with certain safety issues
- *Rodents* will remain important test animals
- *Minipigs (micropigs)* will increasingly been used as alternative non-rodent species to dogs
- There is currently no indication that *ferrets, rabbits or guinea pigs* might become further important alternative non-rodent species in general toxicology

A look into the future – 2

- *Monkeys* will
  - *Not replace dogs or minipigs* in general toxicity testing
  - *Play a role in early toxicity testing* for early entry into man with small amounts of drugs
  - *Remain important* for special issues such as
    - Clarification of questions
    - Increasing confidence in the safety of certain drug candidates

A look into the future – 3

- *Pharmaco-/toxicogenomics will increase in importance*
- *Toxicologists* must be increasingly prepared to draw the attention of medical personnel to potential adverse effects in humans including limitations on dosing and drug-drug interactions (bad example: statin myopathy)

Or in other words…

Not much will move!

Because we are relatively confident with our current animal models and have experience with them


THANK YOU
Curteousy of Andy Meier, Biel-Benken

Appendix

See e.g.

Animal Models in Toxicology, 2nd edition, 2007, Shayne C. Gad (editor)
Taylor & Francis Group, Boca Raton London New York, p. 222

Spontaneous lesions in beagle dogs used in toxicity studies.
Morishima H, Nonoyama T, Sasaki S, Miyajima H.
Jikken Dobutsu. 1990 Apr;39(2):239-48.

Examples of non-neoplastic lesions

Basic change	Organ	Pathology
Degeneration	Kidney	Glomerulonephropathy
	Nerve	Radiculoneuropathy
	Testis / ovary	Atrophy / cysts
	Liver	Steatosis, microcystic degeneration, telangiectasia
Inflammation	Foot	Pododermatitis/arthritis
	Tail	Dermatitis/folliculitis
	Pancreas	Pancreatitis
Proliferation	Liver	Biliary proliferation Altered cell foci/nodules
	Adrenal	Altered cell foci/nodules
	Mammary	Hyperplasia

Examples of neoplastic lesions

- Adrenal pheochromocytoma (especially in males)
- Mammary gland fibroadenoma (especially CD and Wist rats); subcutaneous tumors
- Pancreatic islet cell adenoma
- Pituitary gland adenoma
- Testicular interstitial cell tumors (very high incidence in Fischer rats)
- Thyroid C-cell adenoma

Examples of non-neoplastic lesions – 1

- Various degenerative lesions including e.g. cardiomyopathy, cardiac and cerebral mineralization, nephropathy
- Amyloidosis
- Angiectasis, polyarteritis
- Salivary gland: atrophy, hyperplasia, basophilic foci, cyst
- Ovary, uterus: cysts, hyperplastic changes
- Spleen, liver: extramedullary hematopoiesis
- Lymphoid organs: hyperplasia

Examples of non-neoplastic lesions – 2

- Liver: fatty change, hemosiderosis, microgranulomas, karyo/cytomegaly, nuclear/cellular inclusions, bile duct hyperplasia, oval cell hyperplasia, “fibrosis”,
- Prostate: foci of chronic inflammation
- Lungs: alveolar hyperplasia, inflammatory foci
- Eye: retinal atrophy
- Etc.

Examples of neoplastic lesions

- Bronchiolar/alveolar adenoma/carcinoma
- Lymphoreticular tumors
- Pituitary and various other endocrine organs: hyperplasia and adenomas
- Uterine polyps and other uterine tumors
- Mammary gland carcinomas
- Hepatocellular hyperplasia and tumors
- Vascular tumors
- Harderian gland adenomas
- Etc.

Examples of non-neoplastic lesions

- Amyloidosis
- Calcinosis (connective tissue)
- Multifocal retinal dysplasia
- Polycystic ovaries
- Enteritis (wet tail disease)
- Pneumonia
- Lymphadenitis
- Etc.

Spontaneous tumors are rel. infrequent

- Adrenal adenoma/carcinoma (up to 13%)
- Lymphoreticular neoplasm (~3%)
- Endometrial polyp, carcinoma (each ~3%)
- Adrenal carcinoma (~3%)
- Islet cell adenoma (~3%)
- Papilloma of stomach/vagina (each ~2%)
- Thyroid carcinoma (~2%)
- Etc.

Examples of spontaneous lesions – 1

Susceptible to

- Overgrown teeth
- Scurvy (deficiency of vitamin C)
- Enteritis, often with multifocal inflammatory lesions in the liver
- Infections
  - Lungs, often with alveolar adenomatosis
  - Salivary gland: cytomegaly virus infection
  - Secondary lesions e.g. in the spleen
- Anorexia

Examples of spontaneous lesions – 2

- Heart: interstitial lymphocytic myocarditis
- Lungs: (papillary) adenomas
- Pancreas: acinar cell hyperplasia/ adenoma
- Kidney: chronic interstitial nephritis
- Urinary bladder: cystitis
- Ovary: cysts (also para-ovarian cysts)
- Dystocia with dead fetus frequent
- Myopathy

Examples of spontaneous lesions – 3

- Lymphocytic choriomeningitis
- Pancreas
  - Diabetes mellitus (virus infection?)
  - Islet cell adenoma
- Adrenal: cortical hyperplastic nodules or adenomas
- Various skin lesions including alopecia and dermatitis
- Mammary gland: mastitis. Tumors are relatively frequent
- Etc.

Examples of spontaneous lesions – 1

- Cystic mammary gland hyperplasia often with pituitary adenomas and uterine hyperplasia/epithelial tumors
- Intestinal tract:
  - Plasma cell infiltrations
  - Enteritis (often infectious)
- Liver: cytoplasmic vacuolation
- Rhinitis
- Uterine adenocarcinoma with lung metastases

Examples of spontaneous lesions – 2

- Kidney: lymphoid and macrophage infiltrations
- Urine: cloudiness is normal (crystals)
- Venereal diseases
- Oviduct cysts
- CV system: interstitial myocarditis, arteriosclerosis
- Lymph nodes: lymphosarcoma
- Etc.

Examples of spontaneous lesions – 1

- Infections: Prone for influenza and pneumonitis (can be used as model for influenza and influenza vaccine testing)
- Chronic interstitial nephritis and urinary tract infections
- Adrenal cortex: hyperplasia, adenoma and carcinoma → may lead in middle-aged to older ferrets to endocrinopathy with high estrogen (alopecia, reproductive and behavioral signs)
- Pancreas: tumorous islet cells
- Lymphosarcoma (retrovirus-associated?)

Examples of spontaneous lesions – 2

- Chordoma especially at the tip of the tail
- Skin: sebaceous epithelioma and mast cell tumor
- Estrus-associated aplastic anemia. Can be lethal because of hemorrhage
- Cardiomyopathy with enlarged heart and congestive heart failure
- Prone to stress-related gastric ulcers
- Splenomegaly of unknown cause common in middle-aged to older ferrets
- Etc.

Examples of spontaneous lesions – 1

- Kidney: calcium deposition, tubular vacuolization, inflammatory foci including interstitial nephritis and pyelonephritis
- Cardiovascular system: vacuolation of Purkinje fibers in the heart, peri/polyarteritis, telangiectasia in various organs
- Thymus: cortical atrophy
- Testis: focal atrophy
- Prostate: prostatitis
- CNS: hydrocephalus, chronic focal meningitis

Examples of spontaneous lesions – 2

- Mononuclear cell infiltration in various organs including liver and lungs, partly with microgranuloma formation
- Adrenal gland: vacuolization of the zona glomerulosa
- Stomach: gastritis, microscopic mucosal mineralization
- Intestines: enteritis and colitis
- Liver: hepatocellular lipidosis

Examples of spontaneous lesions – 3

- Pancreas: Ovoid, acidophilic intracytoplasmic inclusions in acinar cells
- Spleen: Gandy-Gamna bodies (= siderofibrotic nodules = firm, nodular discolorations on or within the capsule)
- Cysts in pituitary, thyroid and parathyroid (mostly embryonic remnants)
- Thyroid: idiopathic follicular atrophy and lymphocytic thyroiditis
- Etc.

Examples of spontaneous lesions – 1

Spontaneous pathology relatively rare

- Arteritis in various organs
- Tongue myositis
- Gastric/duodenal mucosal erosions, inflammation in various parts of the intestinal tract
- Chronic cholecystitis, mononuclear infiltrates in the liver
- Inflammation, hemorrhage and fibrosis of the thyroid

Examples of spontaneous lesions – 2

- Degeneration of bone marrow fat cells, hemorrhage in various hematopoietic and lymphoid organs
- Inflammatory foci in skin with edema and hyper/parakeratosis
- Focal skeletal myonecrosis
- Hypoplasia/atrophy of seminiferous tubules with (relative) LC hyperplasia
- Lung: granuloma, macrophages, mineralization
- Renal tubular basophilia, inflammation, mineralization and interstitial fibrosis
- Hemorrhagic syndrome of unknown origin
- Etc.

Examples of spontaneous lesions – 1

- Mineralization in the brain (corpora amylacea)
- Protein inclusions in transitional epithelium (cytokeratin) of the urinary tract
- Macrophages in intestinal villi
- Foci of mononuclear cells e.g. in thyroid, intestine, kidney and liver
- Lymphoid infiltrates in salivary glands, brain, prostate, and other tissues
- Degenerative and in inflammatory changes in liver, kidneys and heart

Examples of spontaneous lesions – 2

- Multinucleated cells in renal pelvis
- Mineralization of adrenal cortico-medullary zone, ovary, and renal papilla
- Herniation of intestinal glands into GALT
- Reticulo-endothelial hyperplasia in spleen
- Lymphoid hyperplasia of the spleen and lymph nodes
- Involution of the thymus
- Extra-medullary hematopoiesis
- Various infections and parasitic diseases

Examples of spontaneous lesions – 3

- Neoplasic changes uncommon (monkeys in toxicity studies generally too young)
  - B-cell lymphoma in case of
    - Infection with SIV: simian immunodeficiency virus, etc.
    - Immunodepressed animals
- In older animals
  - Uterine leiomyoma
  - Intestinal carcinoma
- Etc.