Quantitative Whole-Body Autoradiography (QWBA) Studies Support Drug Discovery and Development

定量整体自显影（QWBA）研究支持药物发现和开发

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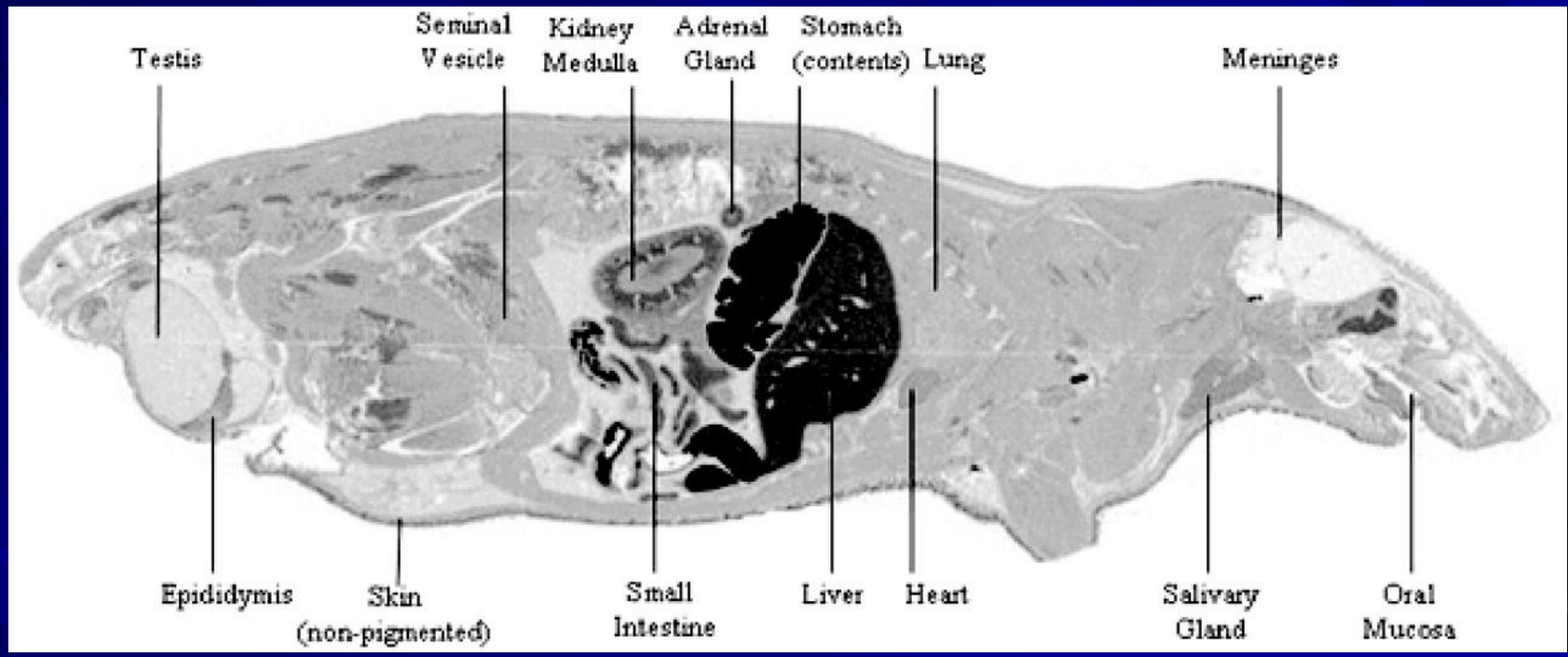
A rat WBA (整体自显影) image



Introduction

What is QWBA:

- **Quantitative Whole-Body Autoradiography**

- **An imaging method to determine the tissue distribution of radiolabeled compounds in lab animals.**

Advantages:

- **Provides a visual whole picture of radioactivity distribution in animals;**

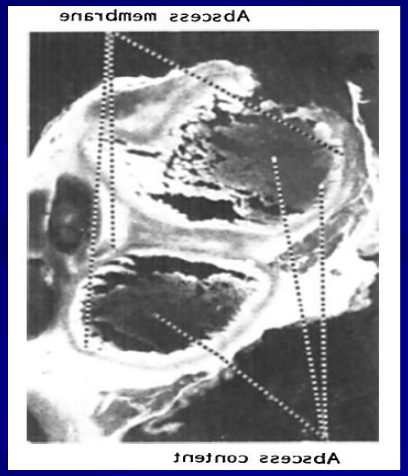
- **Quantitatively determines the radioactivity concentration in most organs and tissues;**

- **Detects potential sites of accumulation and localization in very small tissues.**

History of whole-body autoradiography

- Autoradiography: Niepce (1858), Becquerel (1896)

- Whole Body Autoradiography (WBA): Ullberg (1954)



- Quantitative whole-body autoradiography (QWBA): Luckey G, US Patent, 1975 on the use of phosphor imager, Eastman Kodak

- QWBA finally gained popularity in late 1990's with regulatory acceptance of this technology over the traditional "cut and burn" methods for animal tissue distribution studies.

The Procedures of QWBA

Radiolabelled compound: [^{14}C], [^{3}H], [^{35}S]-drug to animals

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Carcass freezing and embedding

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Sectioning and samples drying

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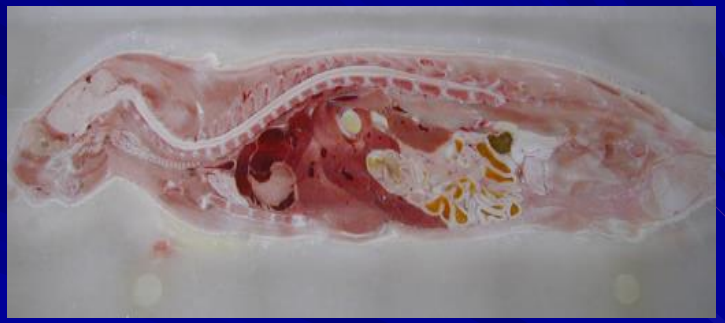
Imaging and quantitation of radioactivity



Why do we do QWBA studies in drug discovery and development ?

- Tissue distribution patterns in animals.
- Determination of drug concentrations in tissues.
- Assessment of the blood brain barrier.
- Affinity to some tissues (such as melanin).
- Dosimetry for human ADME study.

QWBA Studies Discovery And Development

Two basic classes of QWBA studies:

- Regulatory required studies to support drug development:
  - Tissue distribution (QWBA) in pigmented/non-pigmented rats to determine residual tissue radioactivity, used to estimate the radioactive dose of ^{14}C or ^{3}H-drug that can be administered in human study.
  - Detailed tissue distribution in male and female (pregnant/non-pregnant) rats after single and multiple dose to determine organ distribution as well as distribution to milk and to the fetus.
- Exploratory studies help understand questions regarding safety and/or efficacy.

Applications of QWBA in Drug Development

Regulatory required study 1:

QWBA study supports human ^{14}C or ^{3}H ADME study

- **Rats (n=~10, one animal/each time point): ~100 µCi/kg)**
- **Tissue distribution data obtained for most tissues (> 30-40 tissues).**
- **Blood and tissue PK parameters are determined.**
- **Data are used to determine human radiation dosimetry for human ADME study.**

Concentrations of radioactivity in tissues determined by QWBA at specified times after a single oral administration of [^{14}C]drug to rats

	ng Equivalents [^{14}C]drug/g								
	Animal Number (Time Point)								
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Rat 7	Rat 8	Rat 9
Matrix	(0.5 Hours)	(1 Hours)	(4 Hours)	(8 Hours)	(12 Hours)	(24 Hours)	(48 Hours)	(72 Hours)	(168 Hours)
Eye(s)	BLQ	BLQ	297	BLQ	BLQ	ND	ND	ND	ND
Fat (abdominal)	605	983	2150	537	BLQ	ND	ND	ND	ND
Fat (brown)	7060	21600	30600	9050	1910	ND	ND	ND	ND
Harderian gland	2110	6860	28400	12800	5130	BLQ	ND	ND	ND
Intra-orbital lacrimal gland	2120	7440	17100	6330	NR	ND	ND	NR	ND
Kidney cortex	8190	17700	21900	7110	2020	ND	ND	ND	ND
Kidney medulla	5430	13000	16600	5450	1540	ND	ND	ND	ND
Kidney(s)	6860	15300	19400	6610	1780	ND	ND	ND	ND
Large intestine	2120	3910	7560	3290	1850	ND	ND	ND	ND
Liver	65000	91400	93700	47900	16600	608	BLQ	ND	ND
Lung(s)	3710	7730	11800	3610	1120	BLQ	ND	ND	ND
Lymph node(s)	1230	4010	8890	3410	1020	ND	ND	ND	ND
Muscle	1370	3850	7220	2430	741	ND	ND	ND	ND
Myocardium	6810	15400	18200	6160	1920	ND	ND	ND	ND
Nasal turbinates	1110	1770	2480	1090	532	ND	ND	ND	ND
Ovary(ies)	1430[a]	5300[a]	5780[a]	2740[a]	800[a]	ND[a]	ND[a]	ND[a]	ND[a]
Pancreas	5250	15800	22000	6670	1960	BLQ	ND	ND	ND
Pituitary gland	4970	12600	14900	5120	1480	ND	ND	ND	ND
Preputial gland	1510[c]	7050[a, c]	13900[c]	5570[a, c]	3670[c]	1090[c]	451[c]	320[c]	ND
Salivary gland(s)	5520	13800	17400	6380	1750	ND	ND	ND	ND
Skin (nonpigmented)	282	1160	3460	1490	598	ND	ND	ND	ND

Human dosimetry calculation following a radioisotope-labeled compound

$D = (73.8)\ (E_\beta)(C_{max\ (human)})(t_{1/2})$

Where:

D = radioactive exposure (rem);

E_β= average β particle energy for ^{14}C or ^{3}H

$C_{max\ (human)}$ = Maximum radioactive compound concentration (µg eq./g) in humans,

$t_{1/2}$ = biological half-life (days) of ^{14}C or ^{3}H-labeled compound in the rat.

FDA: Limits on Radiation Dose for Adults

Organ or System	Single Dose Sieverts (Rems)	Annual and Total Sieverts (Rems) Dose Sieverts (Rems)
Whole body	0.03 (3)	0.05 (5)
Active blood-forming	0.03 (3)	0.05 (5)
Lens of the eye	0.03 (3)	0.05 (5)
Gonads	0.03 (3)	0.05 (5
Other organs	0.05 (5)	0.15 (15)

Estimated Radiation Dose (rem) in Male Humans Given 100 µCi of [^{14}C]-drug

Adrenal gland	0.00492	0.00717	0.00717	0.00492	0.00492
Blood	0.00142	0.00131	0.00131	0.00142	0.00142
Bone	0.00419	0.00034	0.00034	0.00419	0.00034
Bone marrow	0.03069	0.00273	0.00192	0.03069	0.00192
Brain	0.05434	0.05434	0.05434	0.05434	0.00282
Eye	0.00652	0.00652	0.00055	0.00652	0.00055
Fat	0.05618	0.05618	0.05618	0.05618	0.00299
Kidney	0.00598	0.00661	0.00661	0.00660	0.00661
Large intestine	0.03200	0.03200	0.03200	0.03200	0.03200
Liver	0.01161	0.01161	0.01161	0.01161	0.01161
Lung	0.00246	0.00301	0.00301	0.00246	0.00246
Muscle	0.03278	0.03278	0.03278	0.03278	0.03278
Heart	0.00604	0.06416	0.00604	0.00604	0.00604
Pancreas	0.00407	0.00591	0.00407	0.00407	0.00407
Pituitary gland	0.04972	0.04972	0.00280	0.04972	0.00280
Prostate	0.00286	0.00357	0.00286	0.00286	0.00286
Salivary gland (submaxillary)	0.00323	0.05614	0.05614	0.05614	0.05614
Skin	0.01740	0.01740	0.00130	0.01740	0.00130
Small intestine	0.09210	0.00749	0.00749	0.00749	0.00749
Spinal cord	0.07754	0.07754	0.07754	0.07754	0.00383
Spleen	0.03644	0.00331	0.00221	0.03644	0.00221
Stomach	0.04903	0.00430	0.00326	0.04903	0.00326
Testes	0.03228	0.03228	0.00181	0.03228	0.00181
Thymus	0.02895	0.02895	0.00169	0.02895	0.00169
Thyroid	0.04294	0.04294	0.04294	0.04294	0.04294
Total exposure	0.68572	0.60831	0.41798	0.65462	0.23688

Applications of QWBA in Drug Development

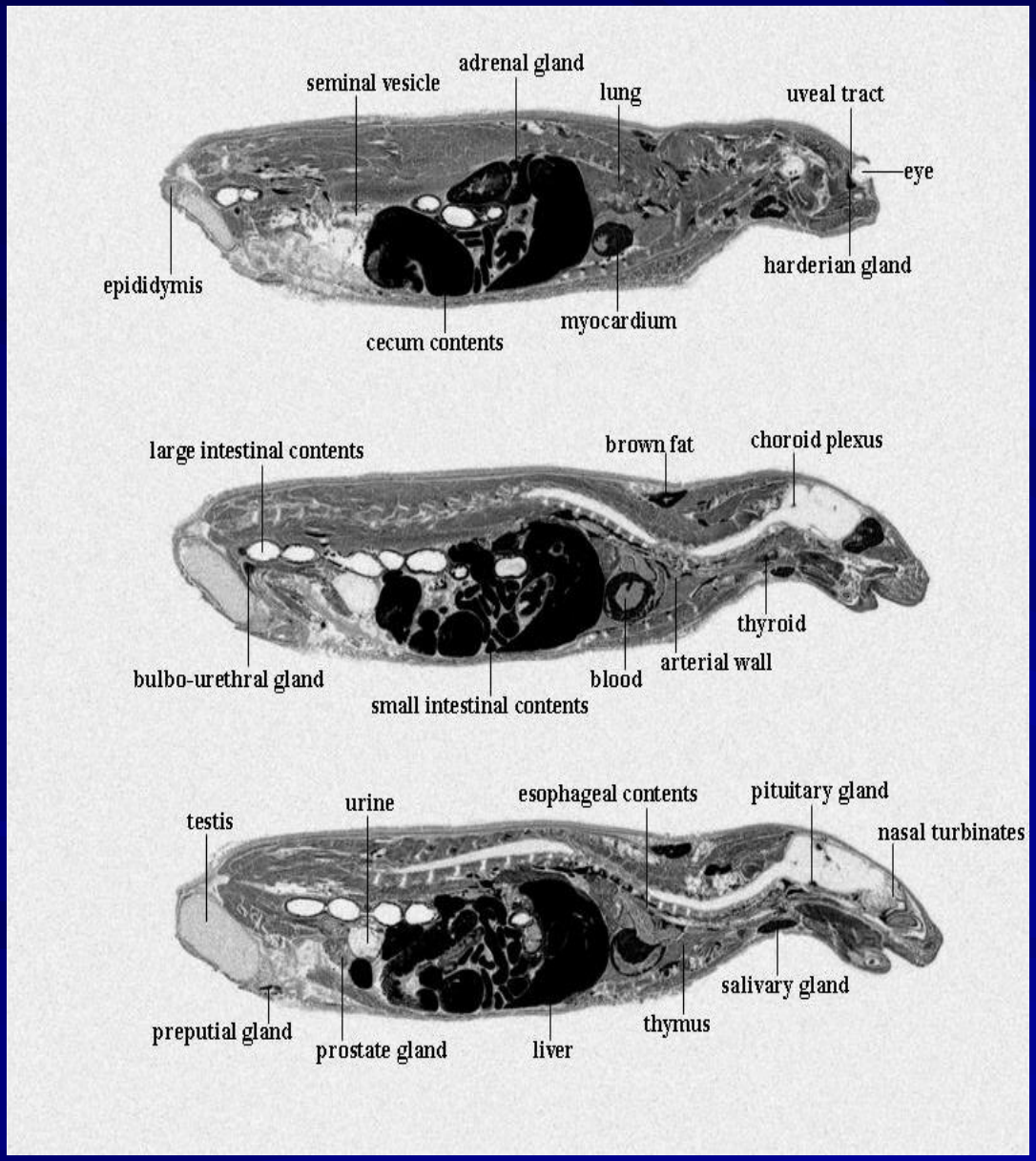
Regulatory required study 2:

QWBA study supports drug NDA

Detailed tissue distribution in male and female (pregnant/non-pregnant) rats after single and multiple dose to determine organ distribution

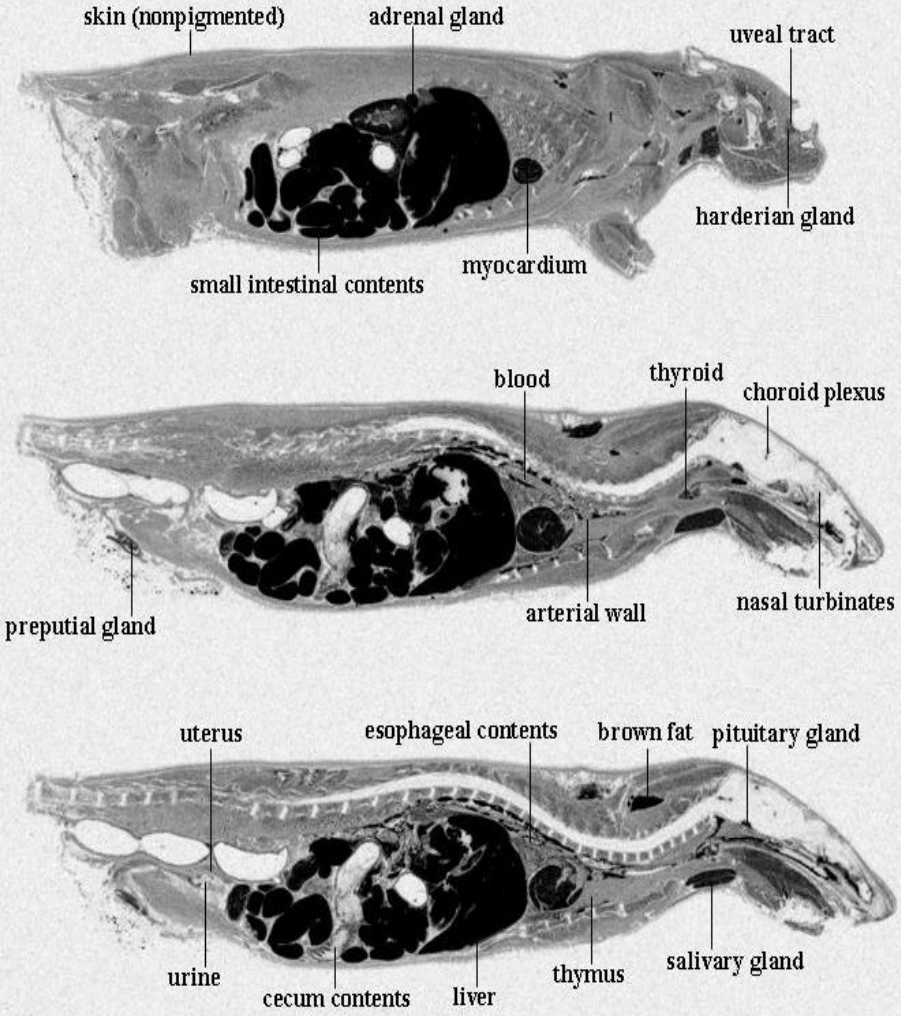
EXAMPLE: TISSUE DISTRIBUTION OF [^{14}C]DRUG X IN RATS

TISSUE DISTRIBUTION OF [^{14}C]DRUG X IN A MALE RAT AFTER A SINGLE ORAL ADMINISTRATION



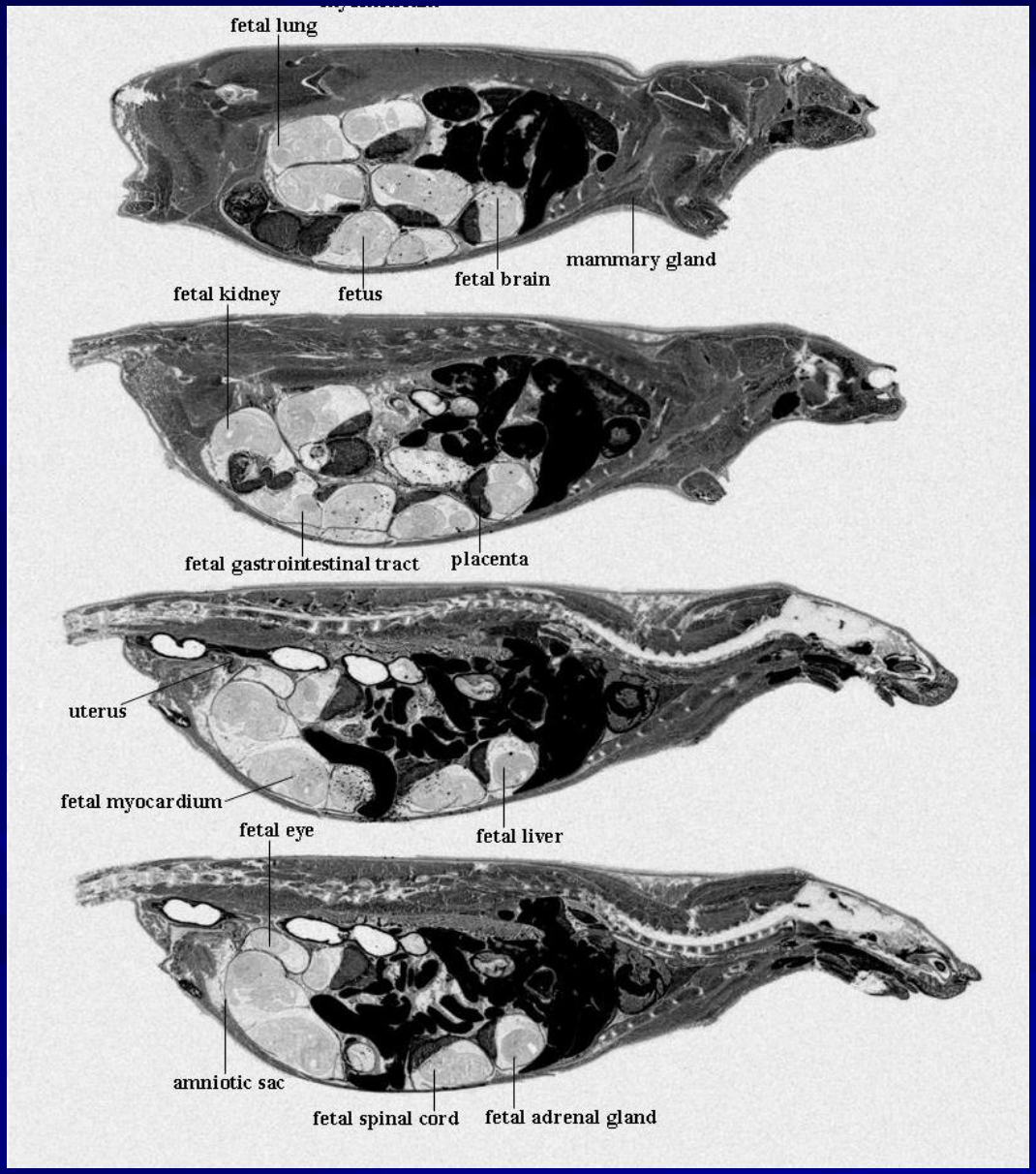
Group 1: male rats.

TISSUE DISTRIBUTION OF [^{14}C]DRUG X IN A FEMALE RAT AFTER A SINGLE ORAL ADMINISTRATION



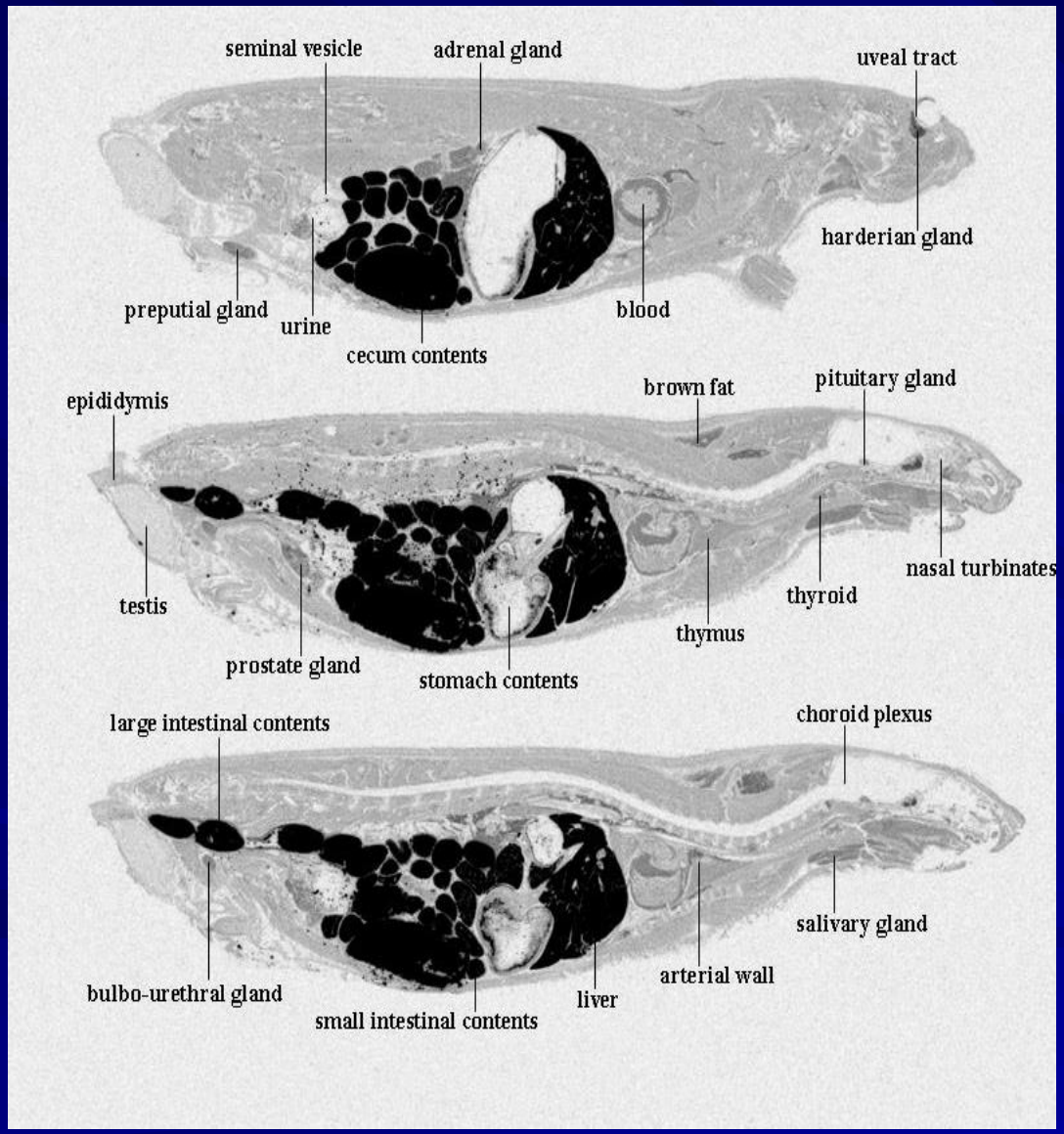
Group 2: female rats.

TISSUE DISTRIBUTION OF [^{14}C]DRUG X IN A PREGNANT RAT AFTER A SINGLE ORAL ADMINISTRATION



Group 3: pregnant rats.

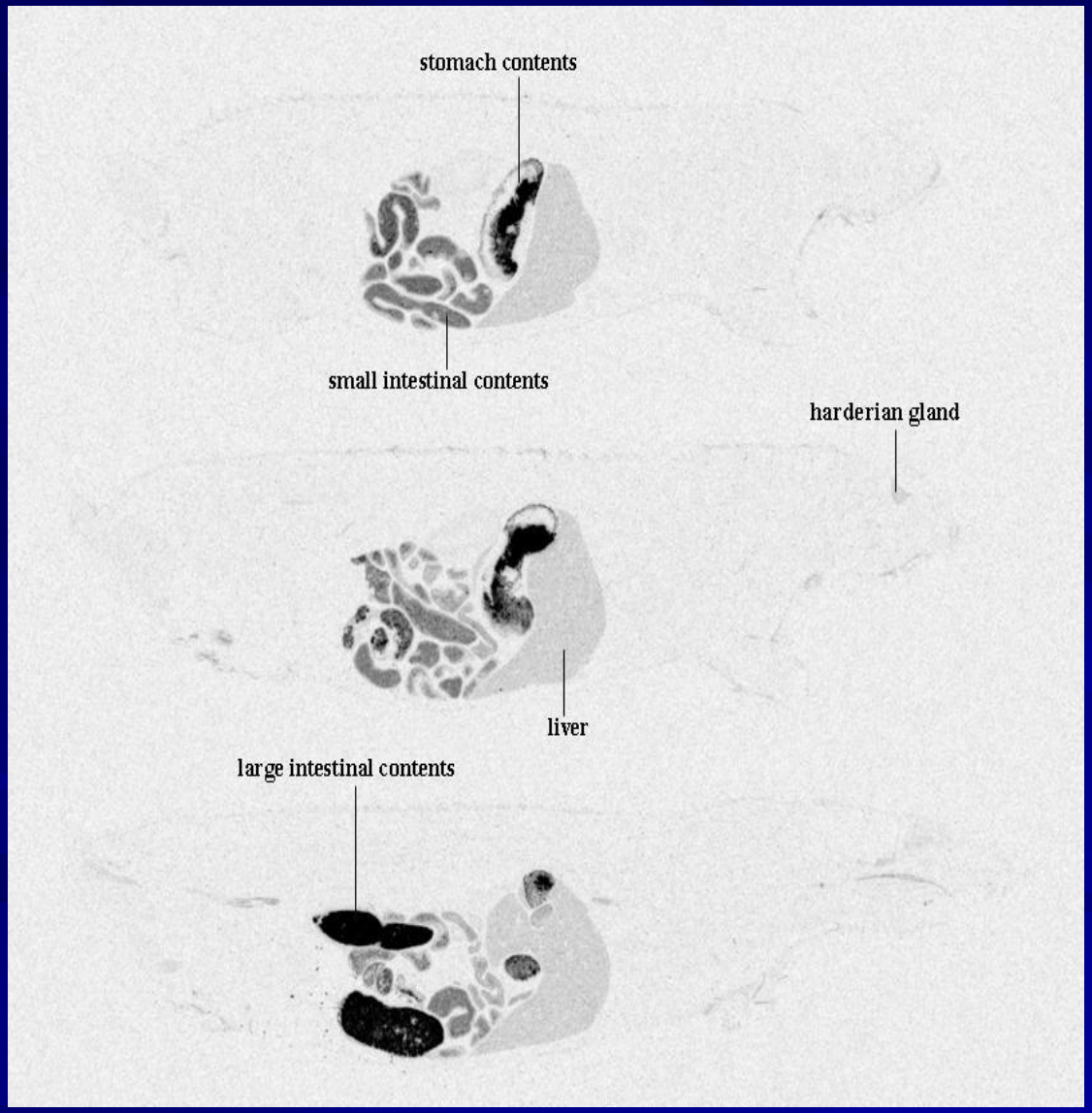
TISSUE DISTRIBUTION OF [^{14}C]DRUG X ONCE DAILY FOR 14 CONSECUTIVE DAYS



Group 4: male rats.

8 hours

TISSUE DISTRIBUTION OF [^{14}C]DRUG X ONCE DAILY FOR 14 CONSECUTIVE DAYS



Group 4: male rats.

24 hours

QWBA: Exploratory studies help understand questions regarding safety and/or efficacy.

- ADME issues
  - Absorption issues; brain penetration, clearance organ
- Pharmacological target tissue localization
  - Target engagement
  - Target tissue distribution
- Toxicological target issues

Discovery application Examples:

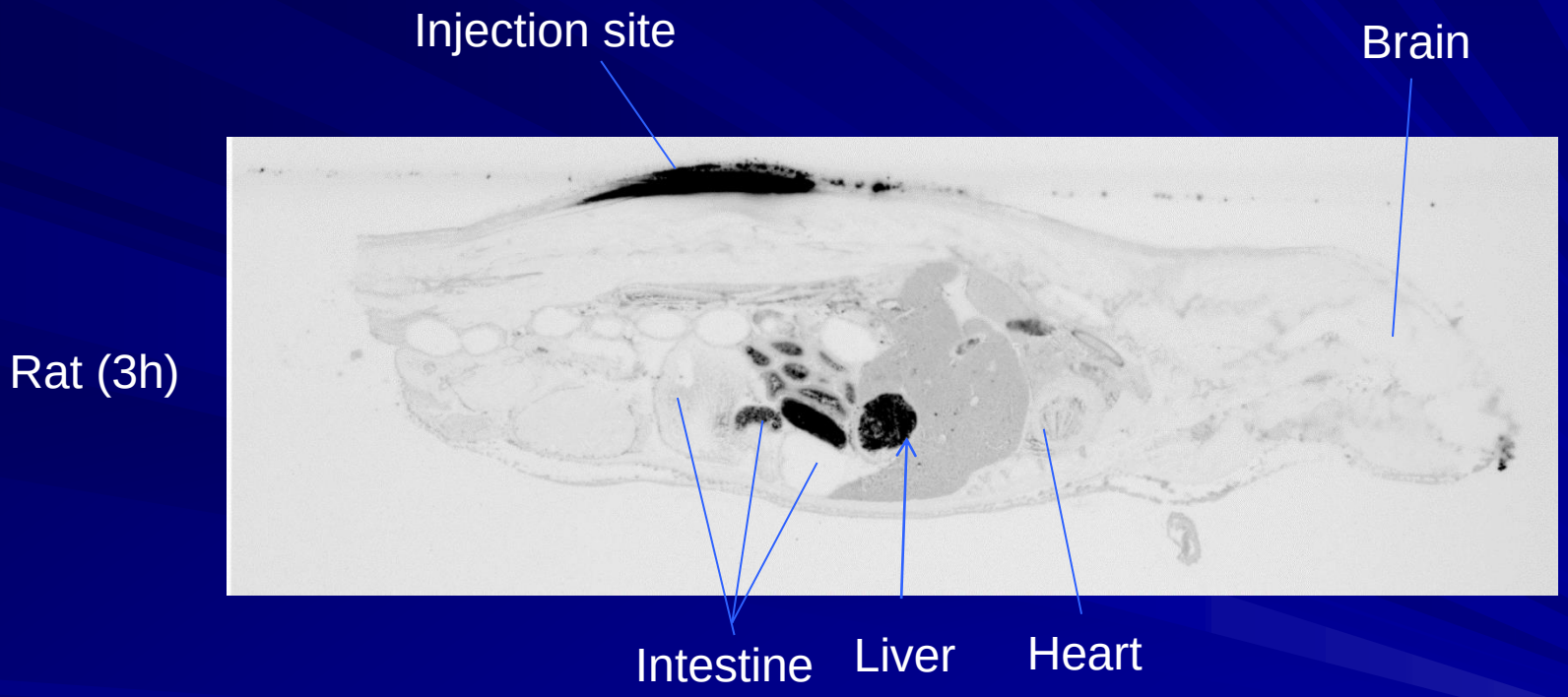
Exploratory formulation development

- Advanced formulation was sought to extend the delivery of compound to allow once weekly dosing

- Compound itself has a relatively short half life

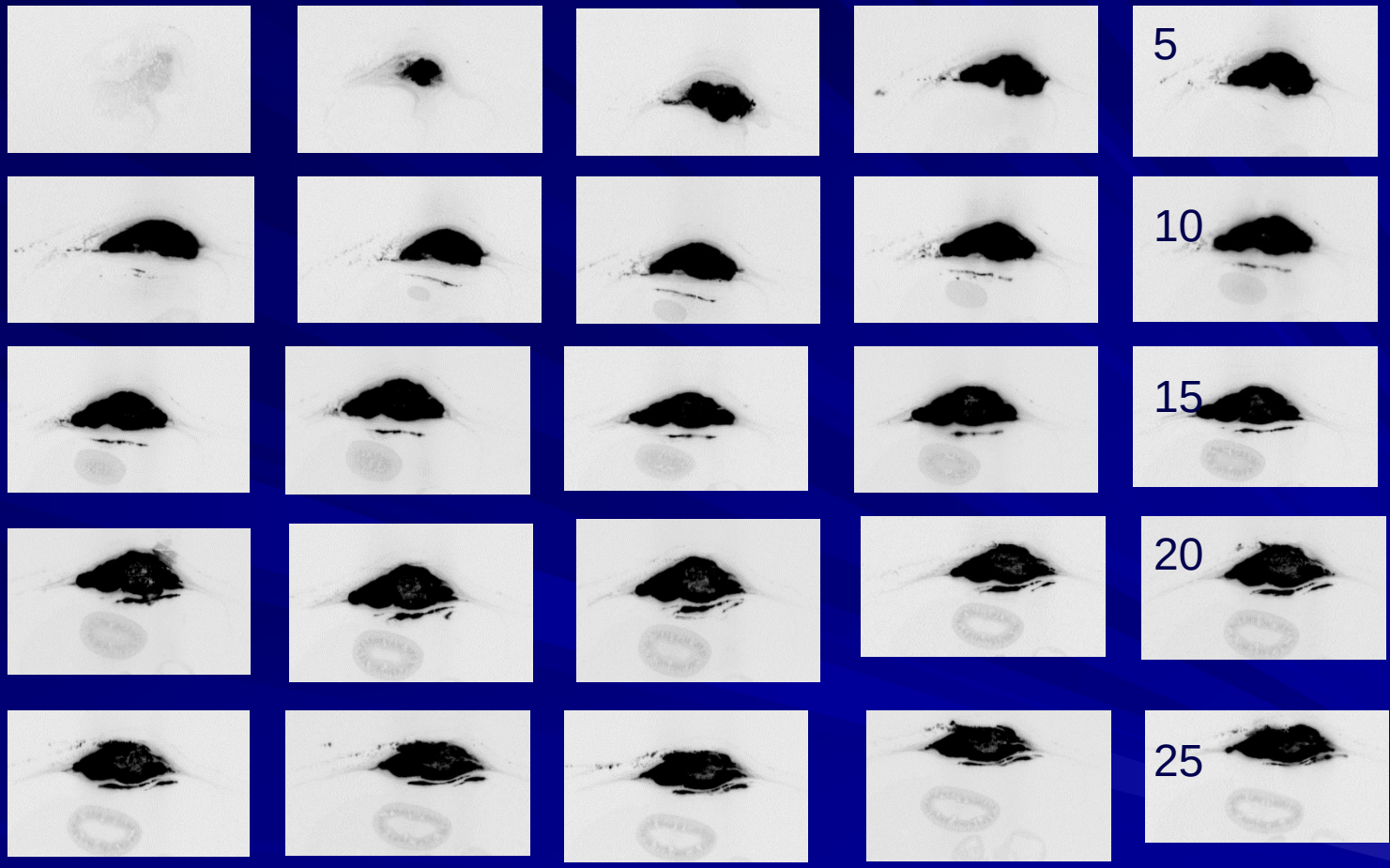
Exploratory Studies – Formulation development

[^{14}C]-drug B was dosed SC at 10 mg/kg to animals for QWBA study



- **SC dosing of various formulations to form depot aiming for sustained delivery of drug**
- **Can we give a better estimate of volume (and perhaps quantity) of drug at the injection site**

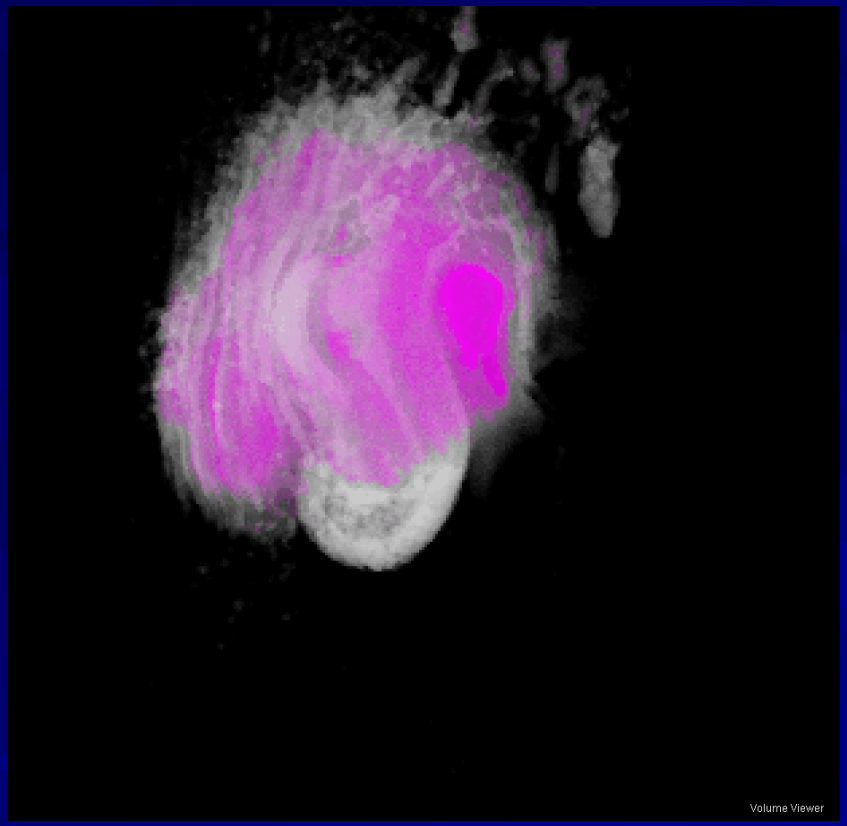
Exploratory Studies – Formulation development



❖ **Serial slices (**the distance between sections is 400 µm) **through injection site 168 hr after sc dose of liquid crystal formulation**

❖ **Used to reconstruct 3D image**

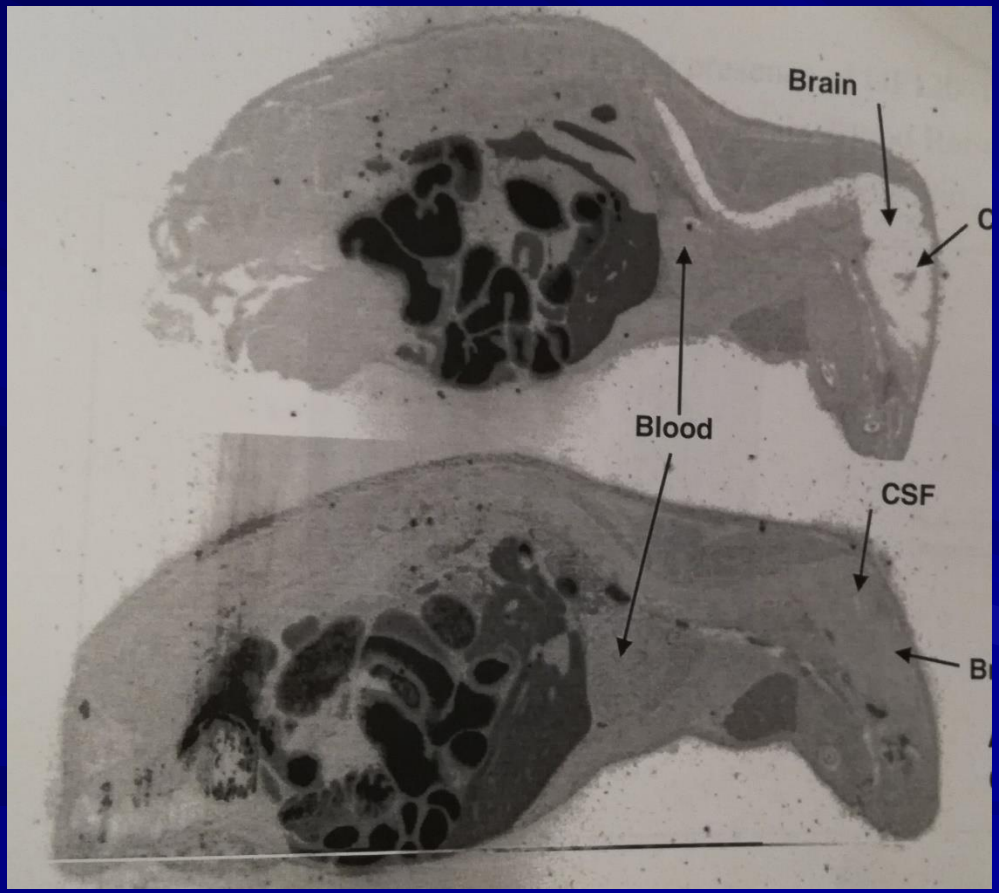
Exploratory Studies – Formulation Development



Useful parameters calculated from these images:

- Volume (weight) of depot over time
- % of dose in depot

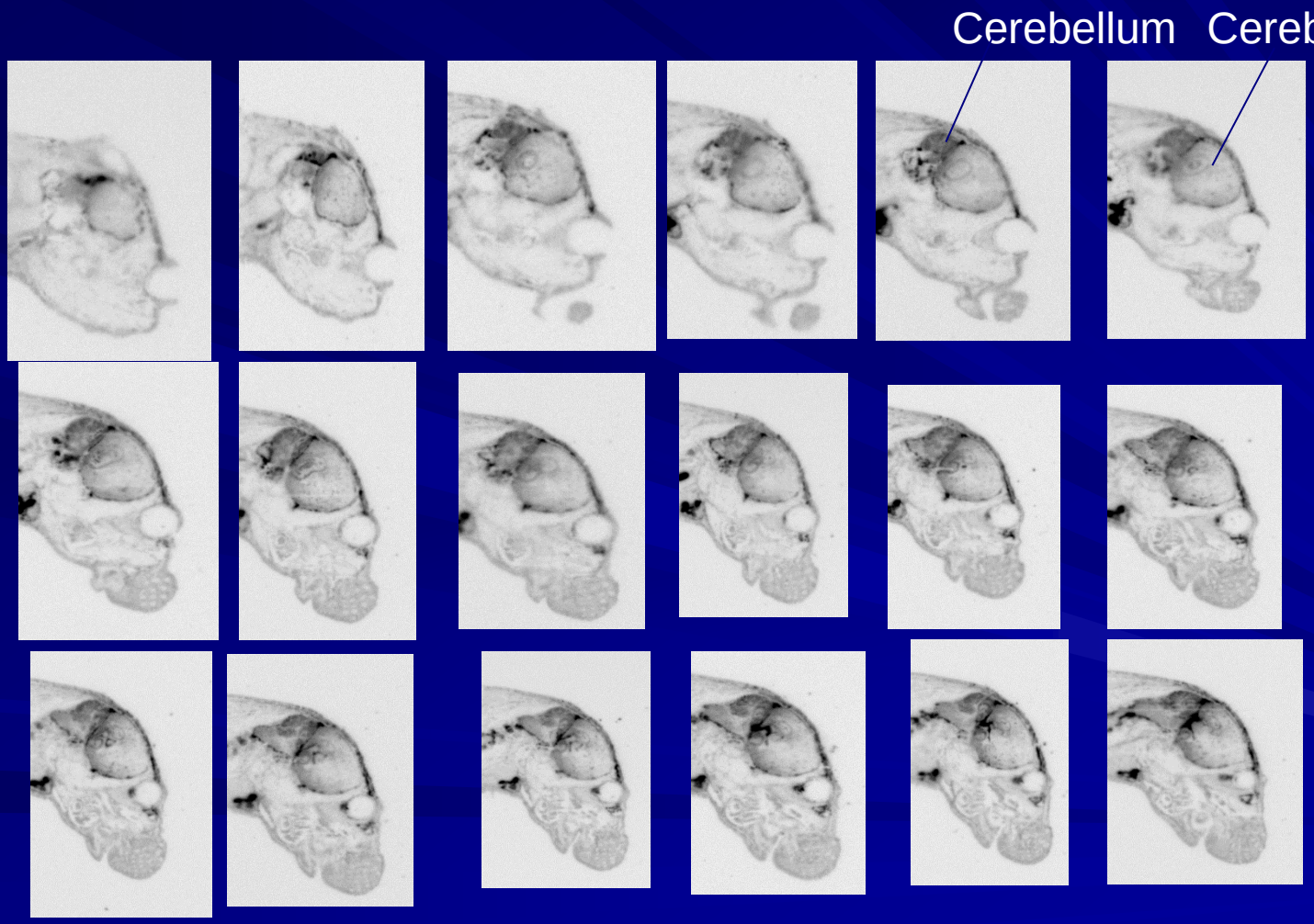
Discovery application example: brain penetration



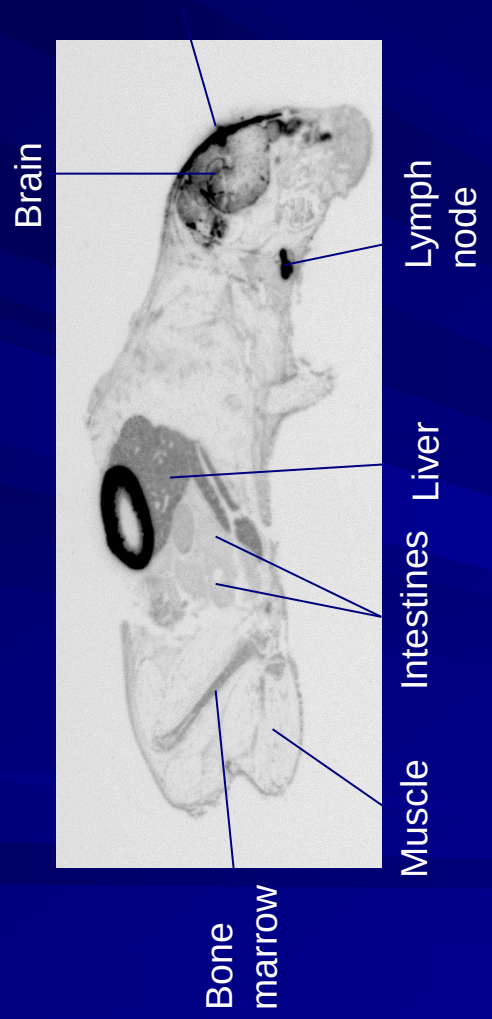
Discovery application example:

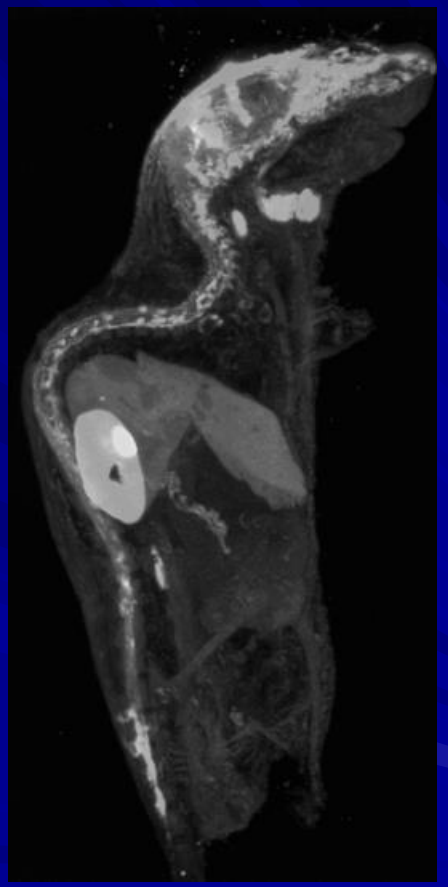
CNS delivery and routes of elimination of oligonucleotide

CNS Delivery of ^{35}S-labeled oligonucleotide



3D rendering of serial 2D QWBA data





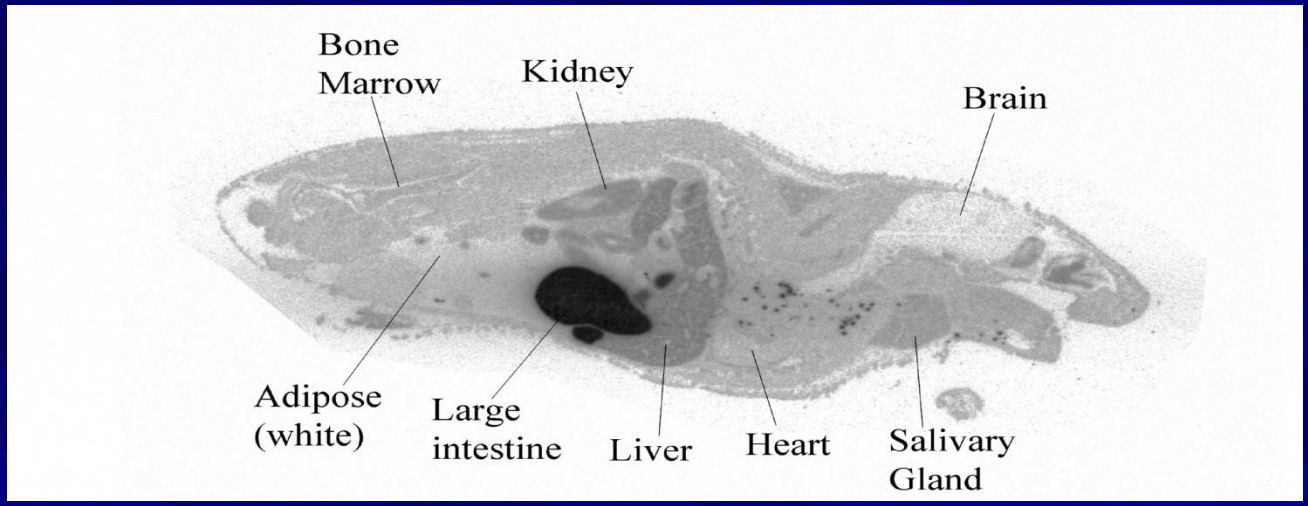
Discovery application Example

Pharmacological target tissue localization (distribution):

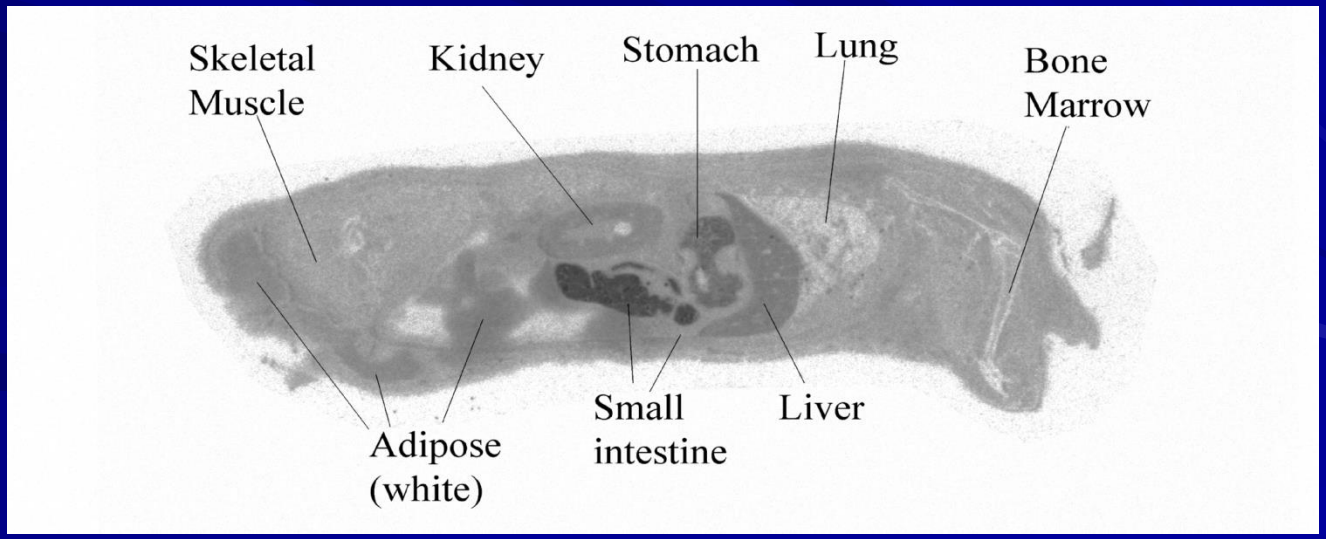
Example: The distribution of [^{14}C]-compound C and [^{14}C]-compound D in adipose tissue and liver of OB mice

Tissue distribution of [^{14}C]-compound C and [^{14}C]-compound D in OB mice (3 h)

[^{14}C]-compound C



[^{14}C]-compound D



The concentration ratio of radioactivity in major mouse tissues

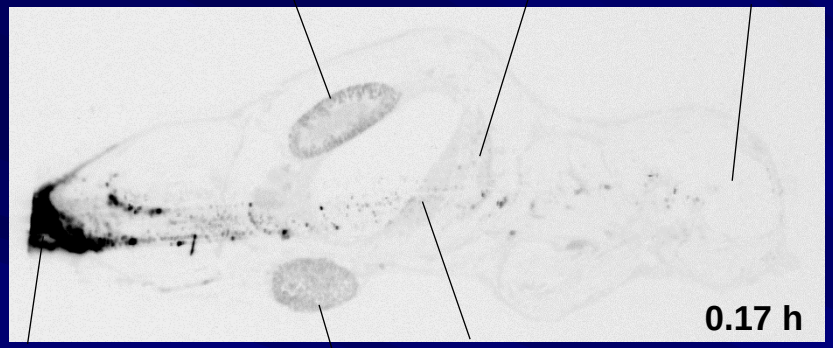
Tissues	Concentration ratio (tissue/blood)							
	[^{14}C]-compound C				[^{14}C]-compound D			
	3 h	8 h	24 h	48 h	3 h	8h	24 h	48 h
Adipose (white)	0.30	0.33	0.33	0.30	3.72	2.66	1.75	0.78
Blood (heart)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Brain (cerebrum)	0.20	0.16	0.29	0.20	0.96	0.82	0.69	0.37
Brain (cerebellum)	0.22	0.16	0.31	0.21	1.11	0.97	0.76	0.38
Heart	1.69	1.62	2.00	1.83	2.17	2.05	1.90	1.18
Kidney (cortex)	3.28	3.08	4.73	4.73	2.81	2.73	4.61	5.78
Kidney (medulla)	1.83	1.54	2.45	2.39	1.29	1.32	1.63	1.48
Liver	4.94	4.39	5.71	9.30	3.88	3.74	5.96	8.11
Spinal cord	0.31	0.18	0.28	0.23	1.04	0.93	0.84	0.44

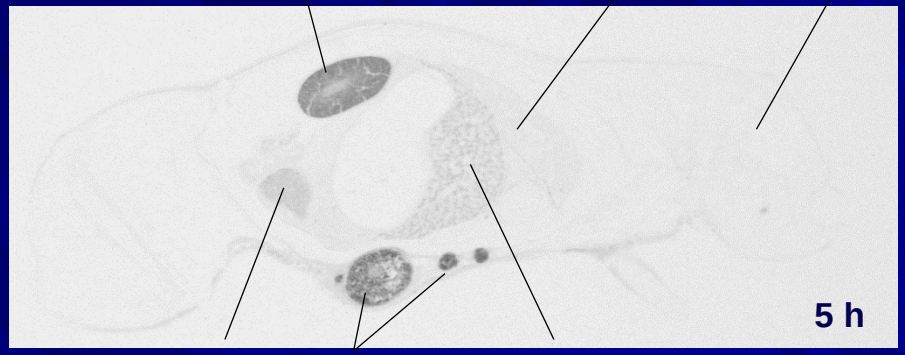
Conclusion: Compound D having a higher adipose exposure could be the reason for the better efficacy.

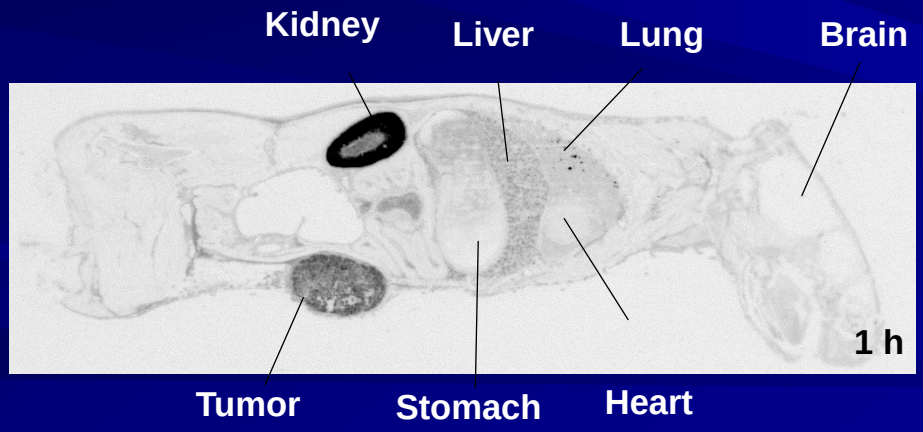
Targeted delivery of antibody drug conjugates

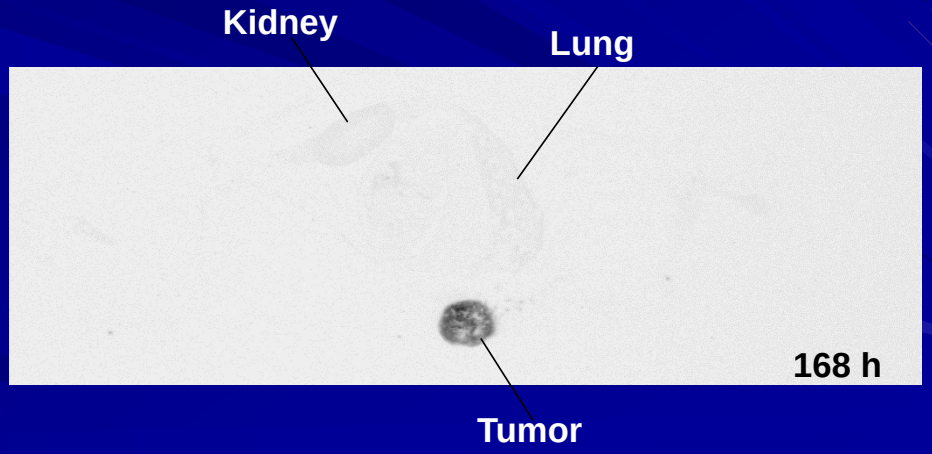
- Antibody drug conjugates (ADC) are designed to deliver cytotoxins to target tissues (e.g., tumor) through specific binding to a cell surface protein target.
- ^{3}H-labeled cytotoxic payload conjugated to non-binding control or tightly binding ADC in tumor bearing mice, and QWBA images were collected at various time points.

Tumor-bearing mice dosed with target binding ADC



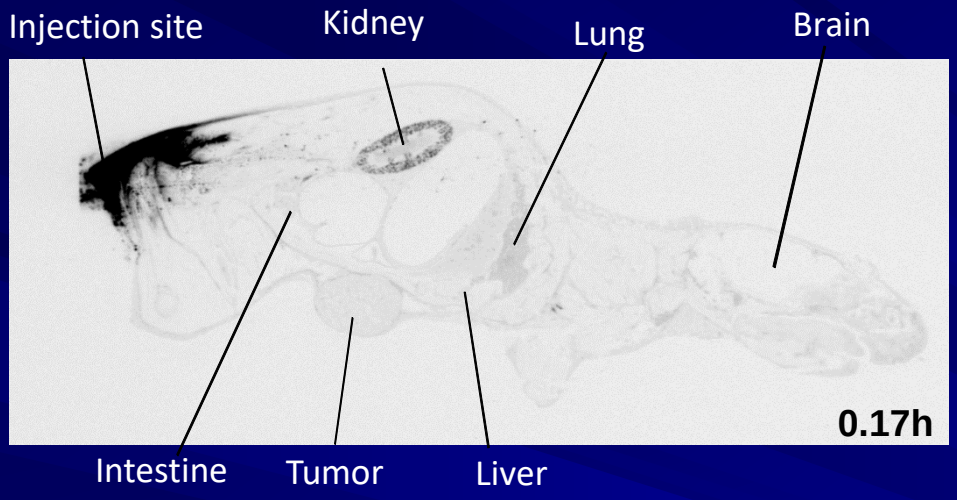


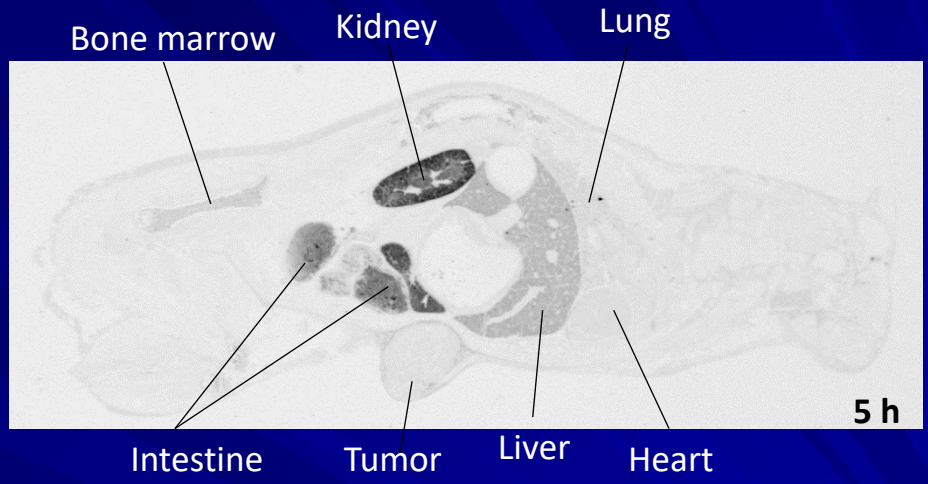


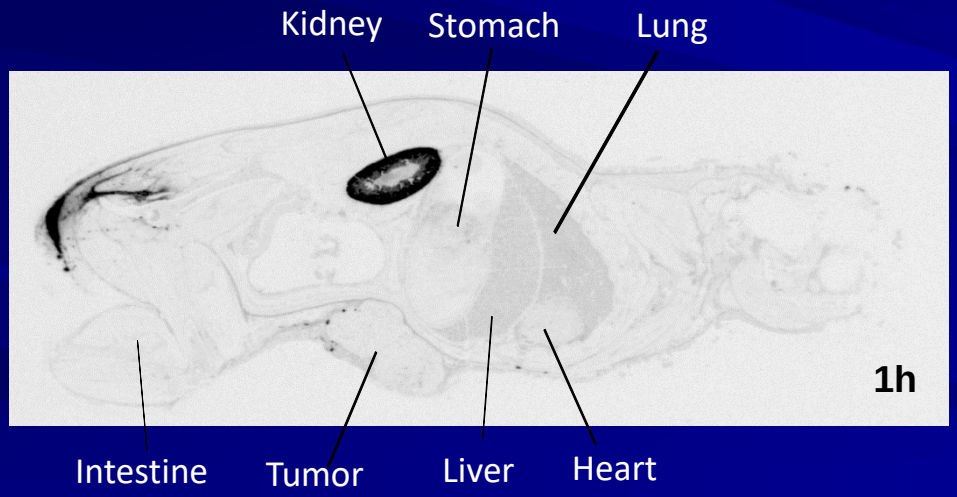


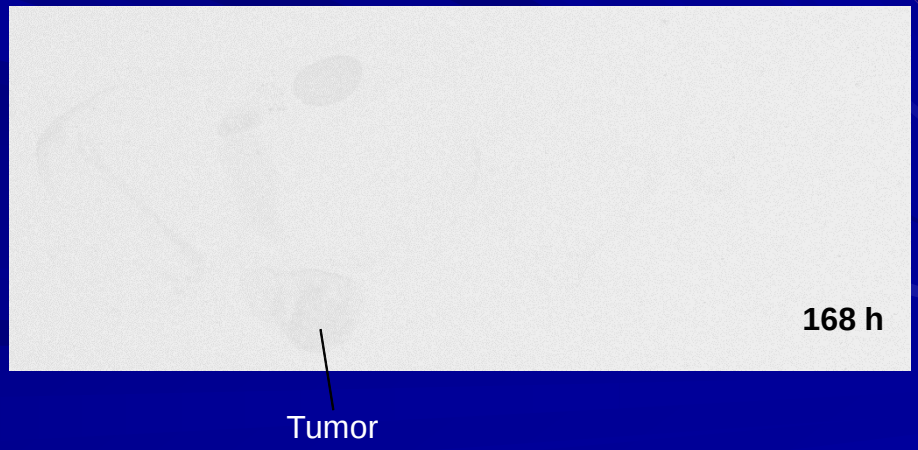
Clear delivery of cytotoxic payload was observed

Tumor-bearing mice dosed with [^{3}H]-labeled non-binding ADC









No specific binding to tumor was observed

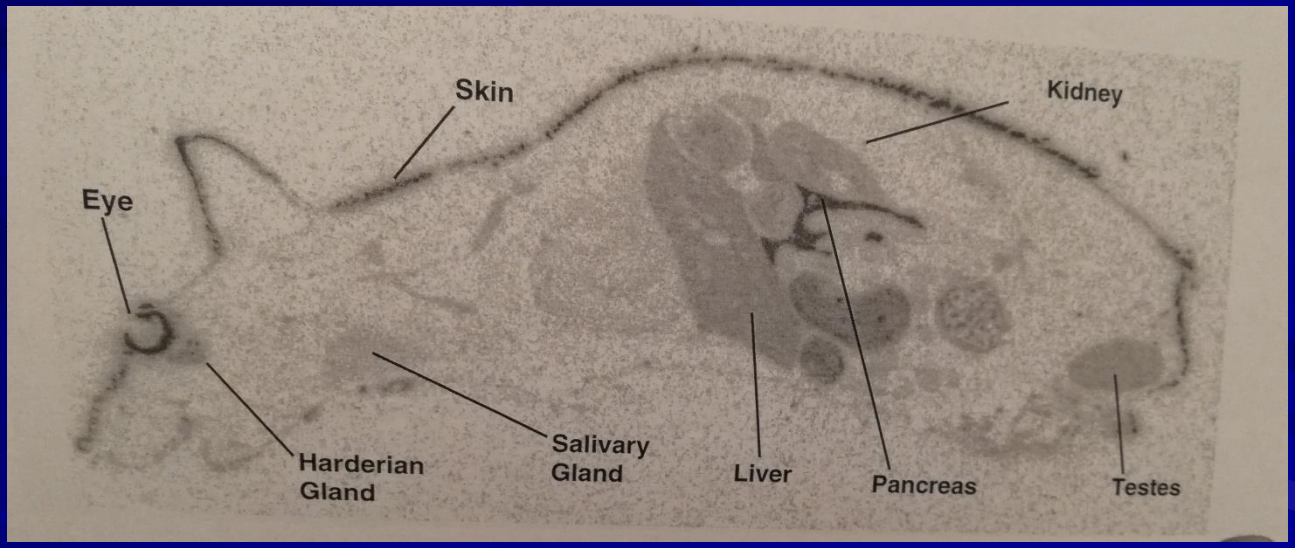
Radioactivity distribution of ADC$^+$ and free payload$^+$ in tumor and liver

Time	Radioactivity in Tumor (%)		Radioactivity in Liver (%)	
	ADC$^+$	Free Payload$^+$	ADC$^+$	Free Payload$^+$
6 h	65.5	34.5	71.4	28.7
24 h	66.1	33.9	58.7	41.4
72 h	50.1	50.0	56.0	44.0
168 h	16.4	83.7	35.9	64.1

Application in toxicological investigation

- Tissue accumulation
- Covalent binding

Tissue binding



Protein covalent binding – the covalent binding of [^{14}C]rofecoxib to the arterial elastin
(*DMD 34:1417–1422, 2006*)

Rofecoxib (VIOXX):

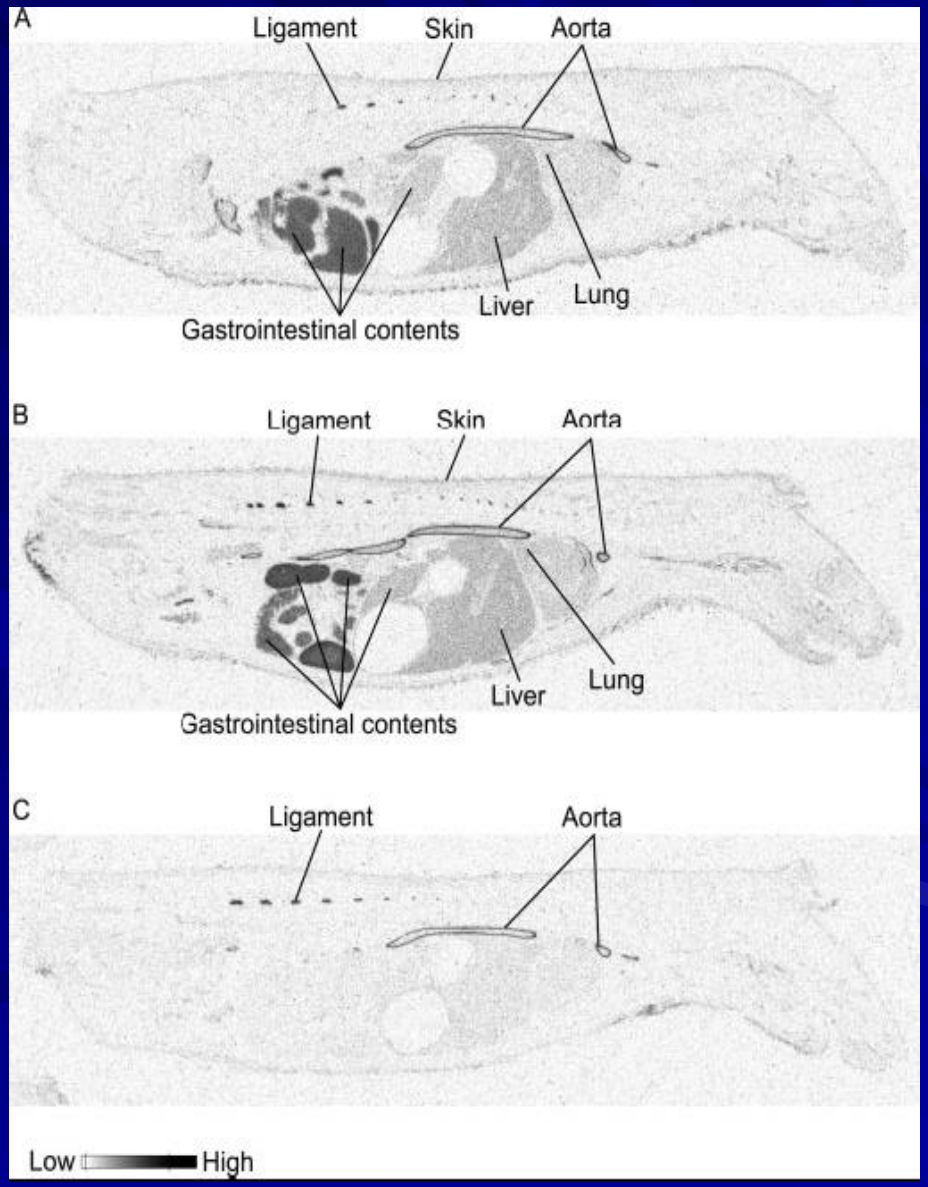
- **A potent and highly selective cyclooxygenase-2 (COX-2) inhibitor that has been widely used as a nonsteroidal anti-inflammatory drug (NSAID).**
- **This drug was withdrawn from the market because of an increased risk of cardiovascular (CV) events**

Whole-body tissue distribution of [^{14}C]rofecoxib (Vioxx) in rats

A: 48 h after 3-day repeated administrations.

B: 48 h after 7-day repeated administrations.

C: 10 days after 7-day repeated administrations.



DMD 34:1417–1422, 2006

Summary

- QWBA is a valuable tool for studying drug distribution
  - Good resolution : organ or tissue level (50-100 µm).
  - Quantification of radioactivity in organs or tissues.

- QWBA can be applied in drug discovery and development to help understand issues related to the tissue localization and targeting of experimental drugs of interest.

- QWBA is best utilized as part of a team effort with input from ADME and analytical scientists, pharmacologists, and toxicologists

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Matt Fronheiser
Wenying Li
Michael Arthur Wallace
John Brailsford
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Ramaswamy Iyer