

# Continuous Infusion Toxicity Studies in Rats: Experiences and Developments at Harlan Laboratories Switzerland

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# Continuous Infusion Toxicity Studies at Harlan Laboratories

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1. Why do you need infusion studies ?
2. History of infusion studies at Harlan Laboratories
3. Development of the cage system
4. Selection of the pump
5. Externalization of the catheter
6. Fine tuning - Validation studies
7. Outlook

## Why infusion studies ?

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### Increasing need for infusion studies over the last 5 to 7 years

- New drugs are frequently proteins
- Cancer treatment (intermittent dosing in treatment cycles)
- Acute treatment with e.g. antibiotics

## Cage System: Tail Cuff Method

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Makrolon type III cage

Catheter is fixed with a metal coil on the base of the tail

Catheter in femoral vein  
(surgery done by Dr.  
Klaus Weber at  
Harlan/RCC)

Used at Harlan/RCC  
until 2002



## Cage System: Tail Cuff Method

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### Problems:

- Bedding material accumulates between metal coil and skin of the tail
- Animals manipulate the metal coil/ try to get rid of it
- Tails are swollen due to mechanical abrasion

### Consequences:

- Local irritations and open wounds on the tail
- Inflammations at the catheter entry site, tail necrosis
- Technique-related loss of animals before the end of the study
- Need to start with 50% more animals than needed for evaluation to compensate for losses

## Cage System: Dilab AccuSampler Cage

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Main difference to the old cage system:

Catheter is exteriorized in the subscapular region of the animal through an implantable skin button (Dacron button)

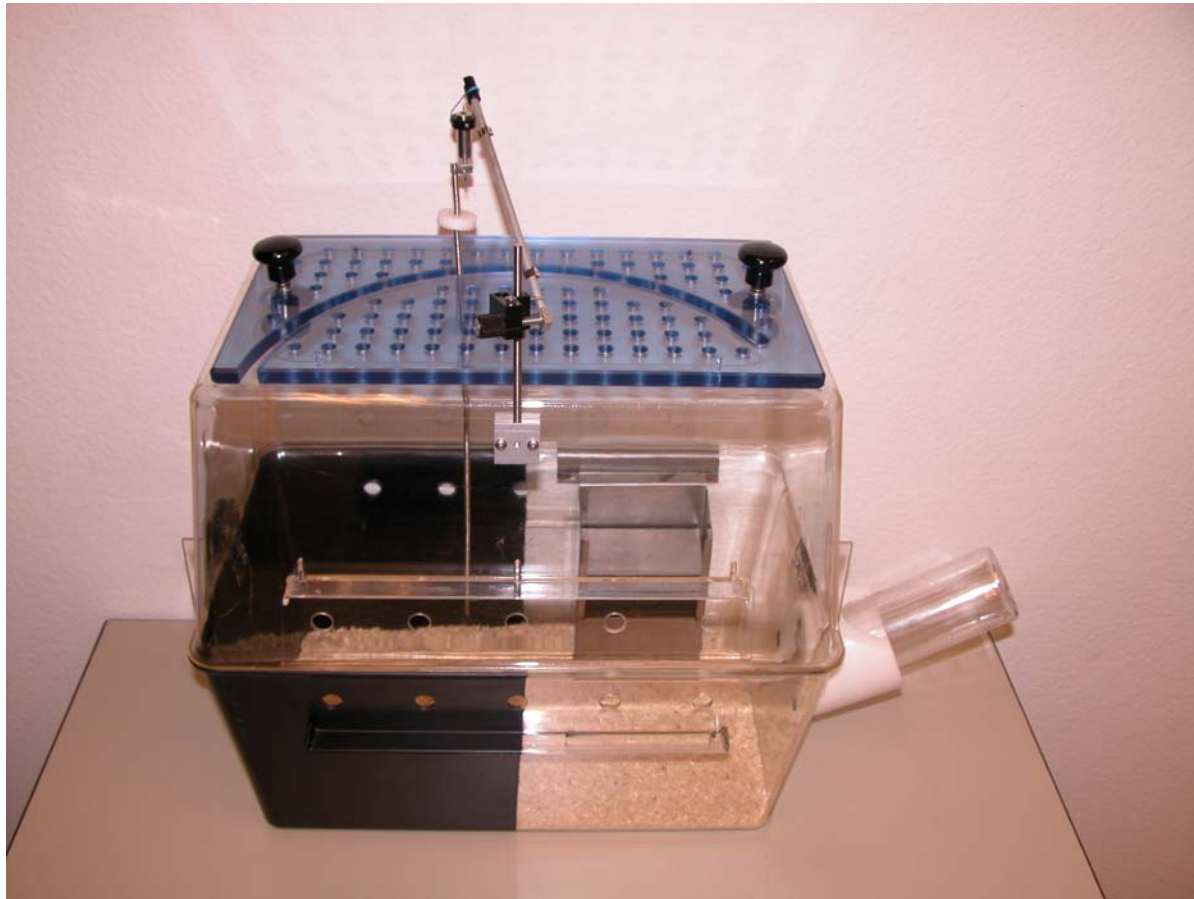
The animals are tethered with a spring coil to a swivel which allows the animal uninhibited movement => better acceptance by the animals

Initial work with this cage highlighted some problems (e.g. “cage tourism”)



## Cage System: Modified Dilab AccuSampler Cage

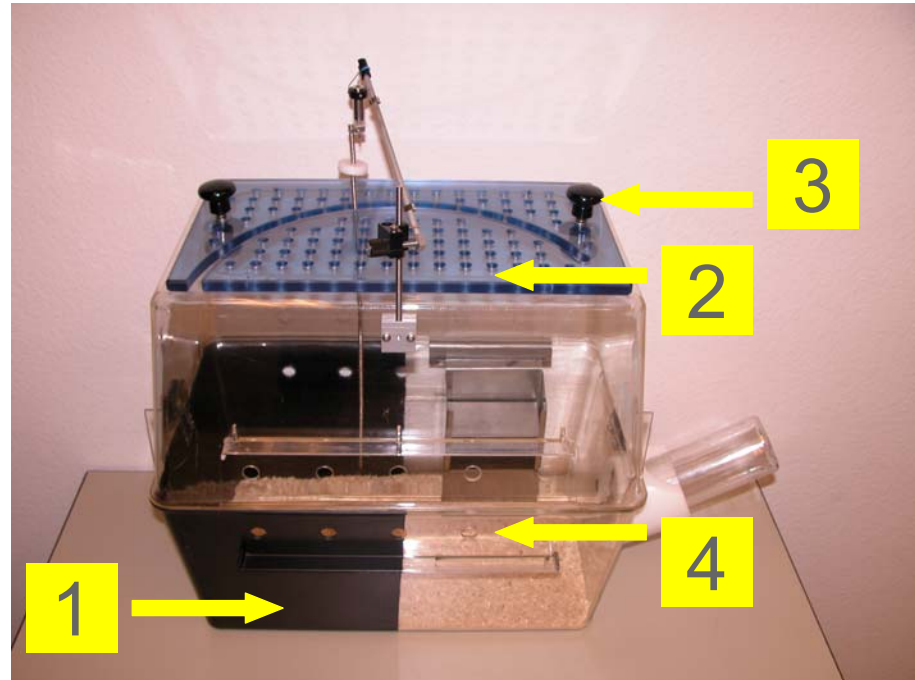
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# Cage System: Modified Dilab AccuSampler Cage

## Modifications:

1. One half of the lower cage part painted in black
2. A ventilated plastic cage top with an arch cut to allow the tether to move freely
3. Fixation buttons
4. Ventilation holes





# Infusion Pump

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## Braun Perfusor Compact S®, Braun GmbH, Germany

- Guarantees definitive start and end points for treatment and recovery periods, an important consideration for GLP studies
- Pump has a back pressure sensor that acoustically indicates possible clotting problems



## An animal room for Infusion Tox Studies at Harlan Laboratories

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## Validation Study No. 1 “4-week infusion”

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**Strain:** Wistar

**Catheterization:** Polyurethane catheter in the *Vena jugularis*

**Catheter attachment:** Dacron button

**Breeding & operation:** By an external supplier

**Treatment period:** 28 days

**Infusion:** 24 hours

	Substance	Dose Volume (ml/kg/h)	No. of Animals
Group 1	5% glucose	1	5♂/5♀
Group 2	5% glucose	2	5♂/5♀
Group 3	0.9% saline	1	5♂/5♀
Group 4	0.9% saline	2	5♂/5♀

## Validation Study No. 1 “4-week infusion”: Results

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The study had to be terminated early because of a **high incidence of premature mortalities** due to

a) **Poor hygienic conditions during surgery**

b) **Incorrect catheterization – in several animals the catheter tip reached the atrium**

The supplier was invited for a discussion of these problems and the surgery procedures were adopted according to the RCC SOP for catheterization

→ A new validation study was initiated

## Validation Study No. 2 – “4-week infusion”

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**Strain:** Sprague Dawley (*considered to be superior to Wistars due to the higher BW at surgery*)

**Catheterization:** Polyurethane catheter in the *Vena femoralis*

**Catheter attachment:** Dacron button

**Breeding & operation:** By an external supplier

**Treatment period:** 29 days

**Infusion:** 24 hours

	Substance	Dose Volume (ml/kg/h)	No. of Animals
Group 1	0.9% saline	1	6♂/6♀
Group 2	0.9% saline	2	6♂/6♀
Group 3	0.9% saline	3	6♂/6♀
Group 4	0.9% saline	4	6♂/6♀

## Validation Study No. 2 – “4-week infusion”

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### Monitoring:

#### During the treatment period:

Viability/ mortality/ clinical signs                      twice daily

Food consumption/ body weights                      twice weekly

#### At the end of the treatment period:

Blood sampling for clinical laboratory investigations

Urine sampling

## Validation Study No. 2 – “4-week infusion”

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Modification of metabolism cages allowed continuous infusion during the urine collection period

One half of the cage painted in black



## Validation Study No. 2 – “4-week infusion”

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Full necropsy of all animals

Macroscopic abnormalities were recorded and various organ weights were determined (according to international guideline)

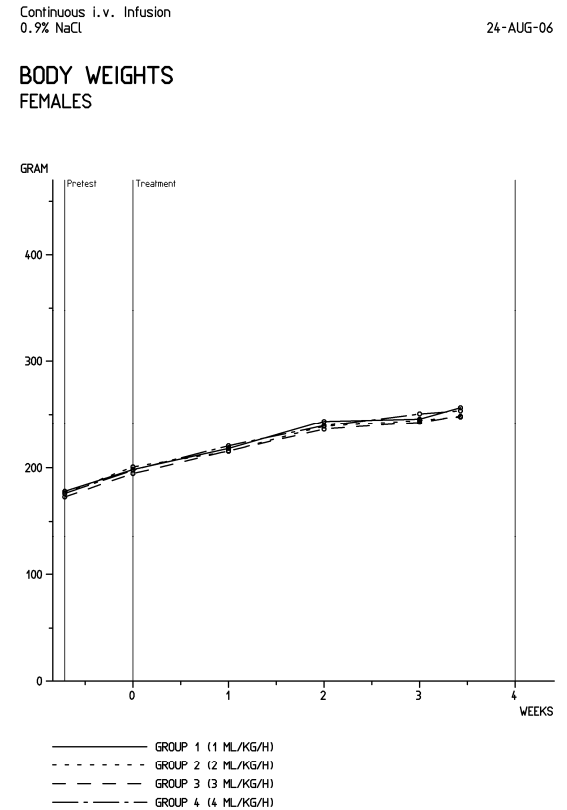
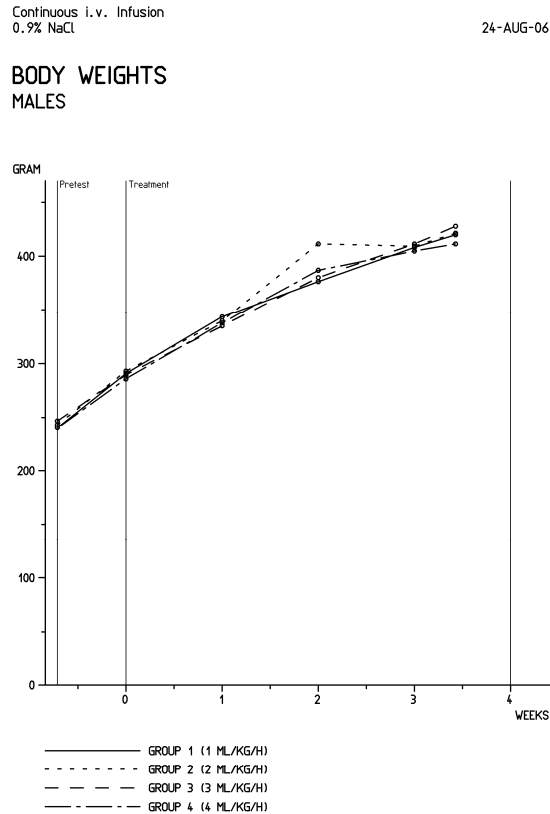
A full set of tissues was collected and slides were prepared for histopathological examination (according to international guideline)



# Validation Study No. 2 – “4-week infusion”: Results

No animals died during the study.

Food consumption and body weight were not affected by the infusion method or the infusion rate.

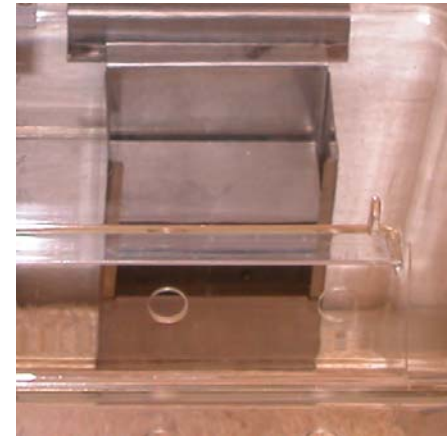


## Validation Study No. 2 – “4-week infusion”: Results

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### Measurement of food consumption

Powder feed was used in a hanging feed box. The box was knocked down by six animals.



For subsequent studies a metal feed rack containing pelleted feed screwed tightly to the cage wall were used.

## Validation Study No. 2 – “4-week infusion”: Results

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### Clinical Laboratory Investigations

→ Most of the measured parameters were within our reference ranges (historical control data)

→ Some values were, unsurprisingly, on or slightly outside our reference ranges from non-catheterized control Sprague Dawley rats.

But: No differences between the groups

## Validation Study No. 2 – “4-week infusion”: Results

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### Pathology

Microscopically, a few animals from all groups showed pathogenesis around the point where the catheter entered the blood vessel:

Phlebitis, periphlebitis and thrombophlebitis.

→ well known and inevitable result of inserting a catheter into a blood vessel and not considered to be an adverse effect

## Validation Study No. 2 – “4-week infusion”: Results

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### Blocking of the catheter

- Occurred 25 times throughout the study
- 19 clots could be removed successfully into a syringe filled with physiological saline
- 6 catheters were treated with human urokinase (2000 IU in 400 µl over 20 min)

The use of urokinase to free clots formed at the catheter tip was considered to be a suitable method with no apparent effect on clinical pathology parameters.

**But:** urokinase treatment can only be performed once per animal (due to possible immune reactions)

## Validation Study No. 2 – “4-week infusion”: Results

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### Catheter attachment

Towards the end of the study the Dacron button detached in 11 animals.

→ The Dacron button was replaced by a harness in these animals

→ → For studies longer than 14 days a harness is preferred.

## Validation Study No. 2 – “4-week infusion”: Results

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### Infusion rates

Infusion rates of 0.9% NaCl at 1, 2 or 3 ml/kg/hour considered suitable (no relevant microscopic changes at the infusion site).

An infusion rate of 0.9% NaCl at 4 ml/kg/hour induced the highest gradings of periphlebitis and thrombophlebitis in single rats at the catheterization site and also some inflammatory processes in organs other than the catheterization site.

→ This infusion rate was not considered to be suitable for such studies.

## Validation Study No. 2 – “4-week infusion”: Conclusions

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- 1.) Infusion studies with Dacron button attachment can be performed for up to 14 days of treatment
- 2.) A dose volume of up to 3 ml/kg/h is recommended
- 3.) Human urokinase can be used to clear clotted catheters
- 4.) Sprague Dawley rats are the better model (... but just for infusion studies)



## Validation Study No. 3 “13-week infusion”

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**Strain:** Sprague Dawley

**Catheterization:** Catheter in the *Vena jugularis*

**Catheter attachment:** Instech Solomon Infusion Harness (Groups 1-3)  
Instech Solomon Vascular Access Harness (Group 4)

**Breeding & operation:** Harlan Laboratories, B.V., The Netherlands

**Treatment period:** 13 weeks

**Infusion:** 24 hours (4 hours)

	Substance	Dose Volume (ml/kg/h)	No. of Animals	Catheter	Infusion
Group 1	0.9% saline	3	10♂	polyurethane	24 hours
Group 2	0.9% saline	3	10♂	silicon	24 hours
Group 3	0.9% saline	3	10♂	silicon round tip	24 hours
Group 4	0.9% saline	3	10♂	silicon round tip	4 hours/day

Initial Set Up

## Validation Study No. 3 “13-week infusion”

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**Groups 1 - 3**

**Instech Solomon  
Infusion Harness:**

Tube to the pump is  
permanently connected to  
the harness

## Validation Study No. 3 “13-week infusion”

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### Group 4

### Instech Solomon Vascular Access Harness (VAH):

Plug / socket principle

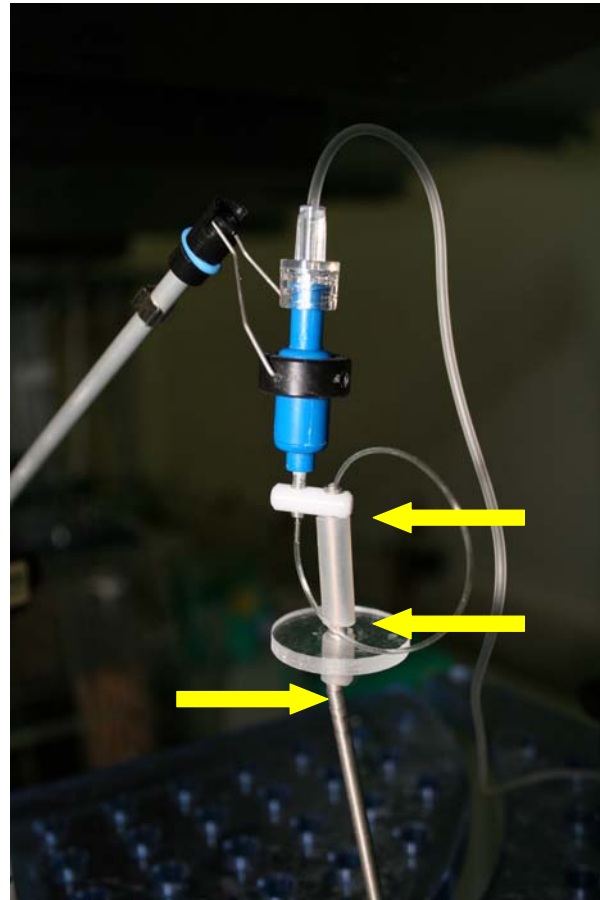


## Validation Study No. 3 “13-week infusion”: Results

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General observation:  
animals with harnesses  
are more active than  
animals with Dacron  
button fixation during the  
first two weeks (Dacron  
button is fixed on the neck  
muscles).

Rapid body shaking led to  
forces impacting on the  
whole catheter system:  
catheter damage



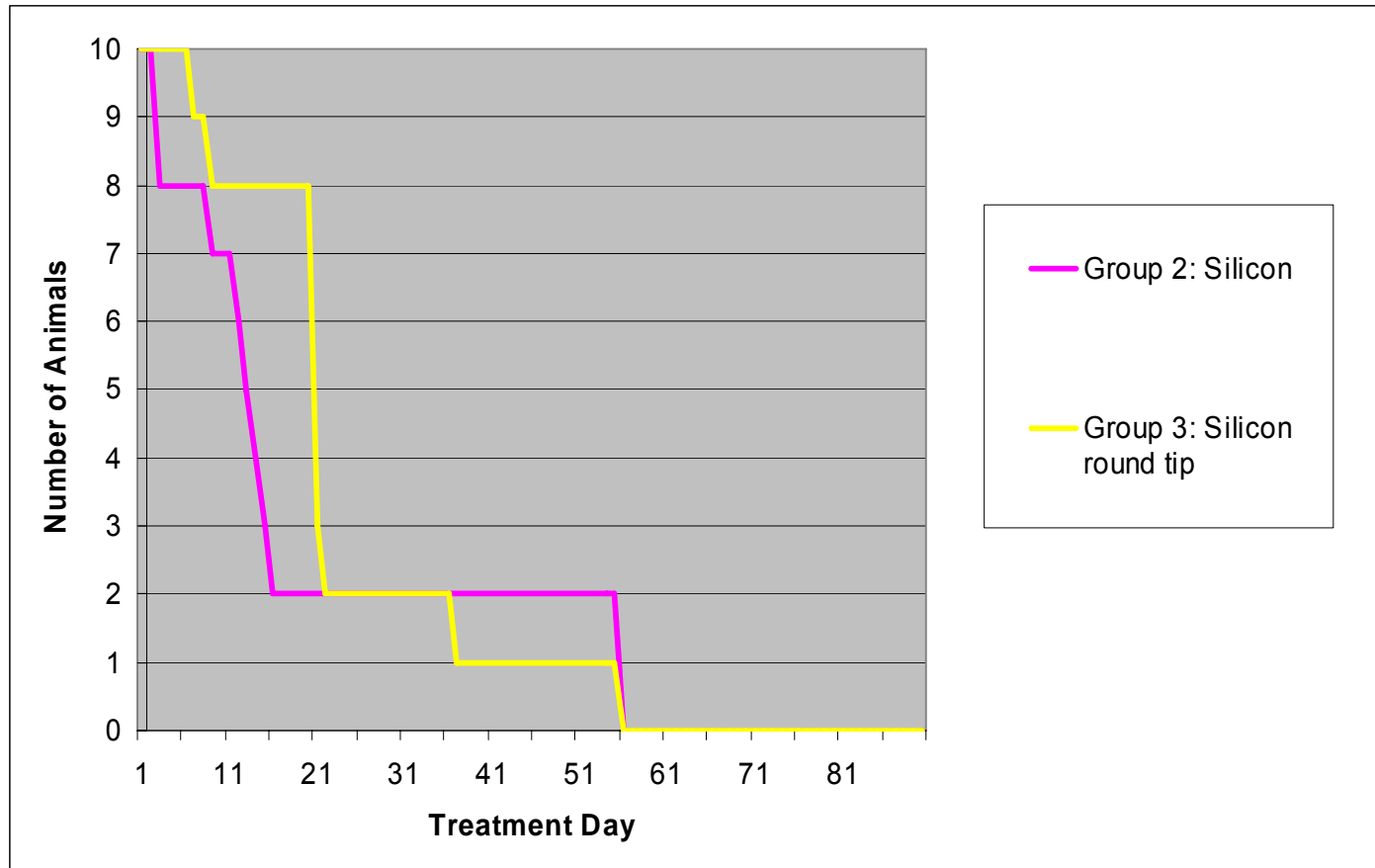
## Validation Study No. 3 “13-week infusion”: Enrichment

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Paper strips enabling nest building and hiding from light during the light phase were used to calm the animals.

## Validation Study No. 3 “13-week infusion”: Results



## Validation Study No. 3 “13-week infusion”: Results

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### Groups 2 and 3 (animals with silicon catheters):

→ No animal reached the end of the treatment period

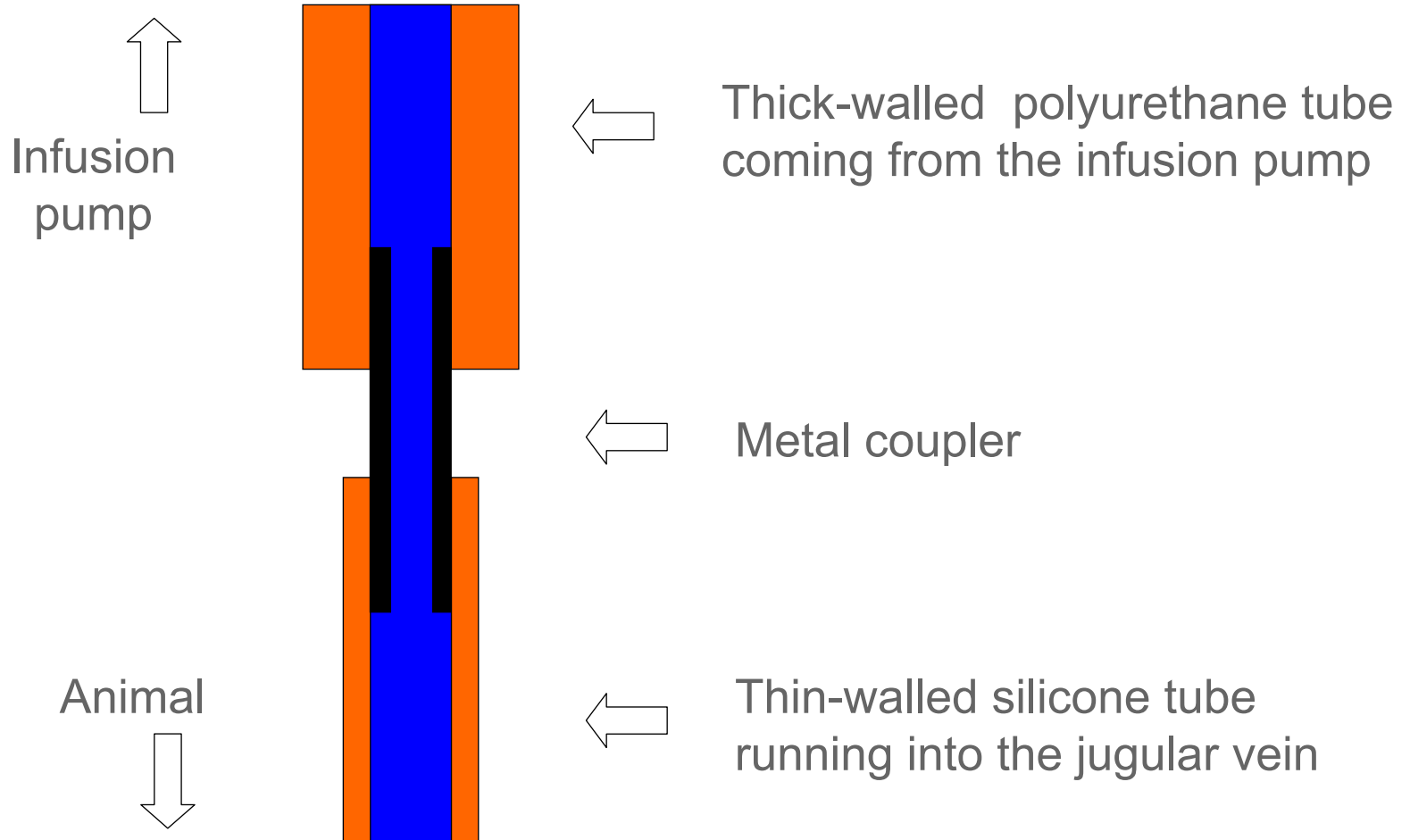
#### Reason for the termination of animals

- 1.) Swellings near catheter entry site – 8/20 animals\*
- 2.) Catheter ripped out of vein – 5/20 animals
- 3.) Spontaneous death – 3/20 animals\*
- 4.) **Catheter detachment – 2/20 animals (see next slides)**
- 5.) General bad condition – 2/20 animals\*

\* (Reasons to be evaluated based on histopathology data)

## Validation Study No. 3 “13-week infusion”: Detachment problem

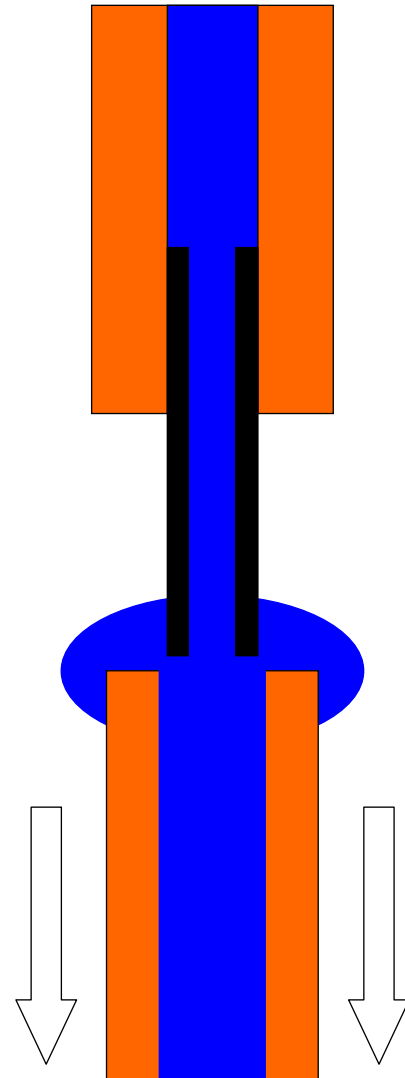
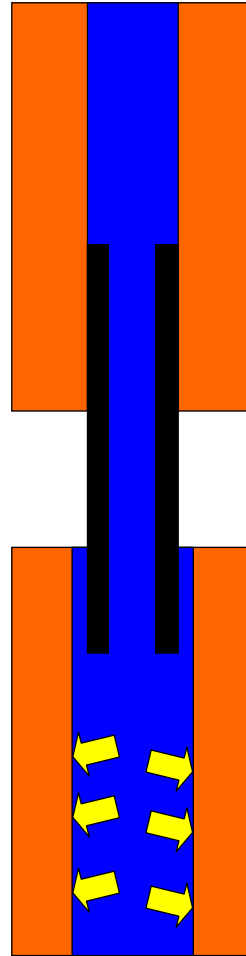
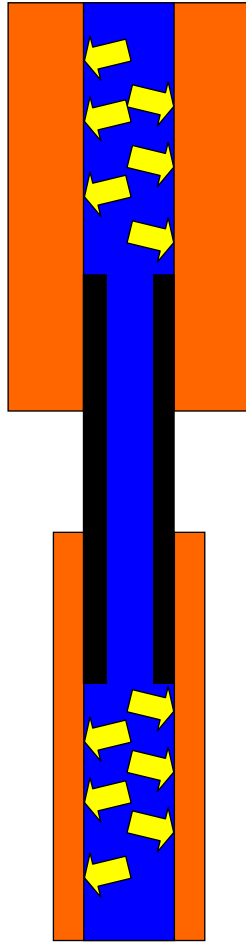
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# Validation Study No. 3 “13-week infusion”: Detachment problem

Hydrostatic pressure



## Validation Study No. 3 “13-week infusion”: Detachment problem

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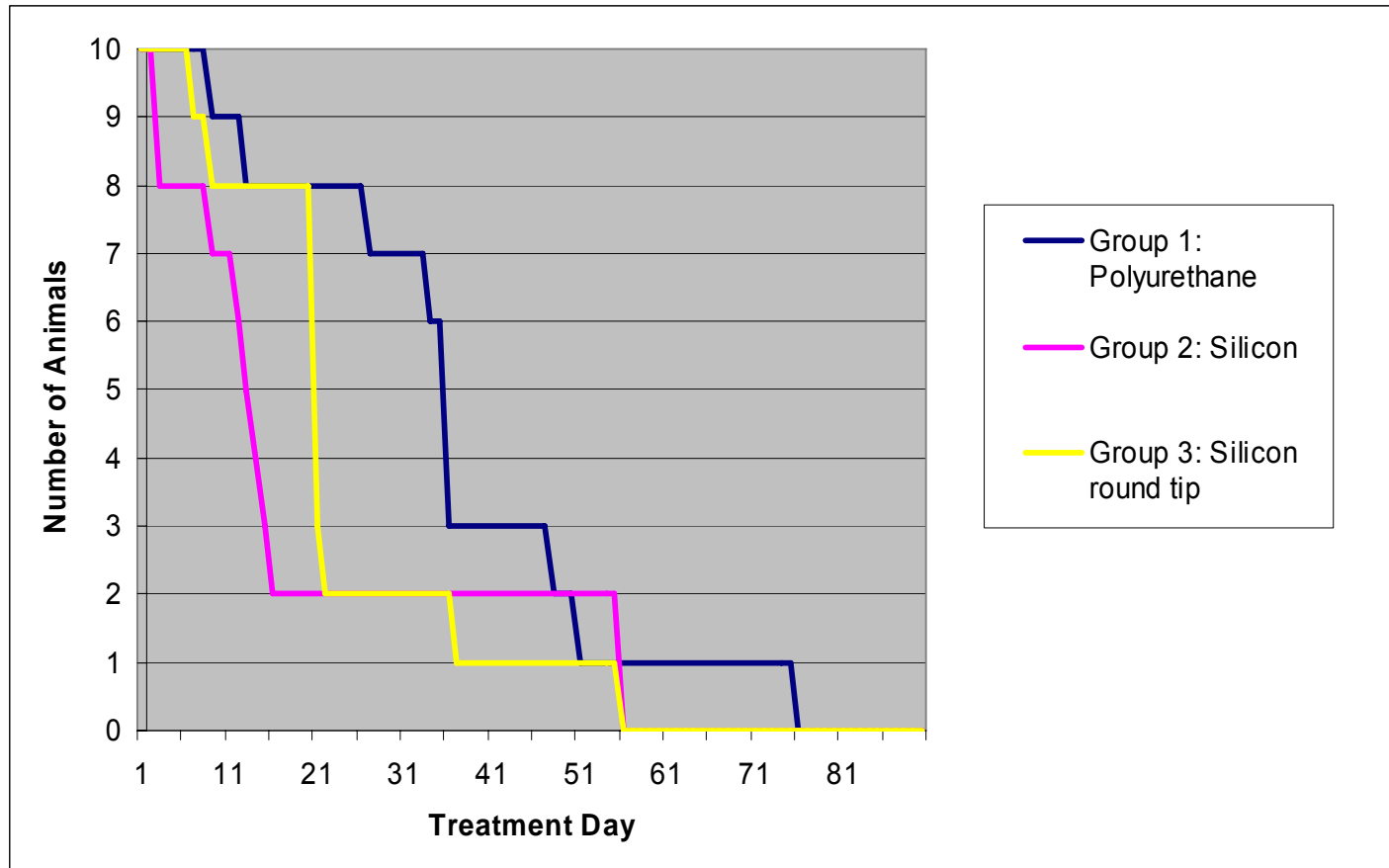
In only 2/20 animals “catheter detachment” was the proximate cause of terminating the animal. However “catheter detachment” occurred in a total of 15/20 animals – in some animals more than once.

→ A surgical operation was necessary - animals had to be anaesthetized and the harness detached. The surgeon had to “search” for the catheter end between muscle and skin of the animal and reconnect it to the metal coupler.

→→ additional stress for the animal

→→→ the “catheter detachment” maybe directly or indirectly related to other causes which made the termination of animals necessary

## Validation Study No. 3 “13-week infusion”: Results



## Validation Study No. 3 “13-week infusion”: Results

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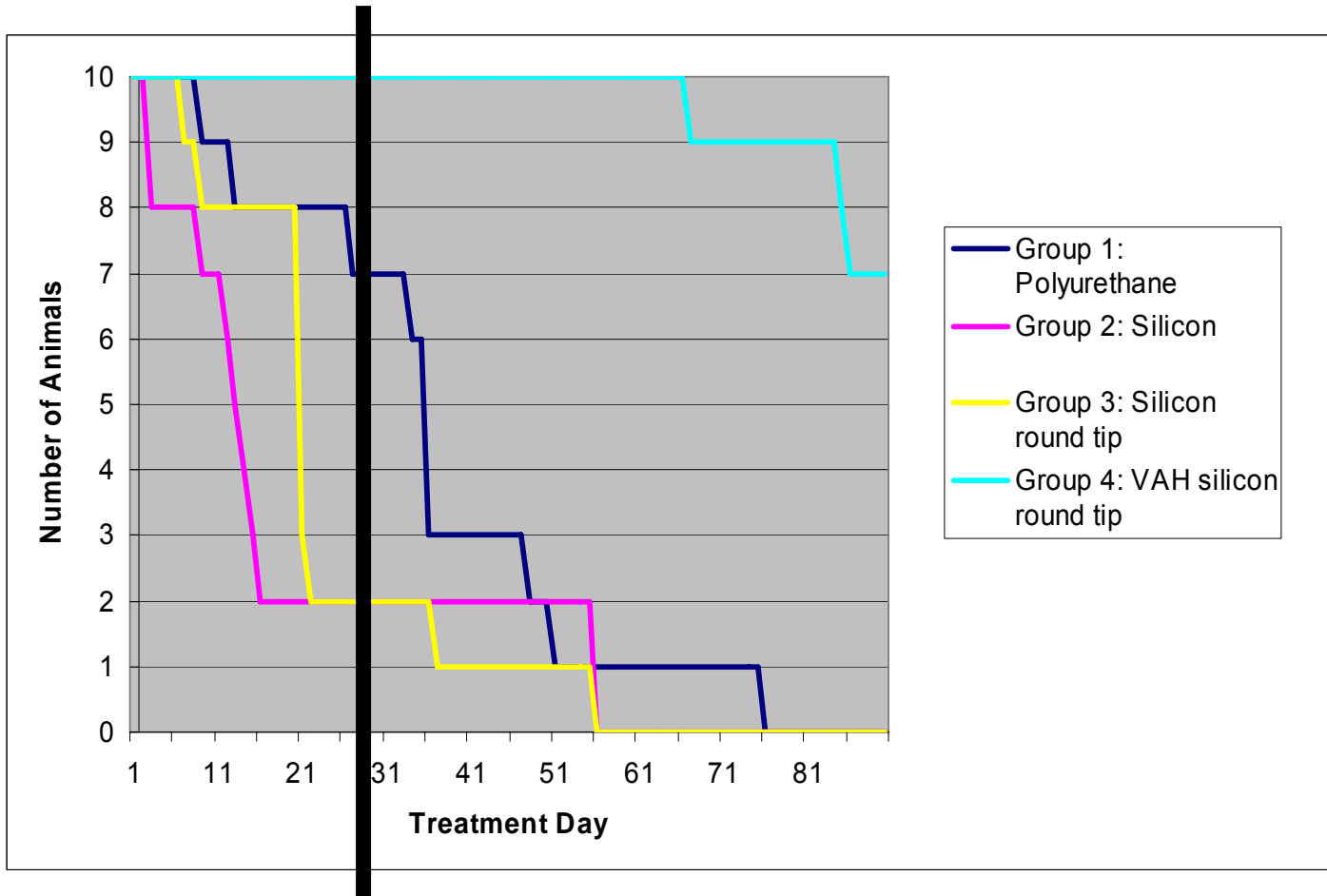
### Group 1 (animals with polyurethane catheters):

→ No animal reached the end of the treatment period

#### Reason for the termination of animals

- 1.) Swellings near catheter entry site – 4/10 animals
- 2.) Catheter ripped out of vein – 4/10 animals
- 3.) General bad condition – 1/10 animals
- 4.) Catheter detachment – 1/10 animals

# Validation Study No. 3 “13-week infusion”: Results



## Validation Study No. 3 “13-week infusion”: Results

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### Group 4 (animals with Vascular Access Harness):

→7/10 reached the end of the treatment period (90 days)

Termination of animals 1st animal – day 66

2nd animal – day 84

3rd animal – day 85

### Reason for the termination of animals

1.) Catheter ripped out of vein – 2/10 animals

2.) Defective catheter – 1/10 animals

## Validation Study No. 3 “13-week infusion”: Conclusions I

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→ Cause of the catheter detachment was identified too late, no animals in groups 1, 2 or 3 reached the planned necropsy date

→ Vascular Access Harness (VAH) proved to be best solution: Animals can be disconnected for recording of body weights and blood sampling, VAH seems to be more resistant to sudden movements of animals

→ No occlusions of the catheters occurred during the disconnected periods in animals with VAH (catheters were not flushed during the disconnected time)

**However: animals in groups 1 – 3 were infused over 24 hours, animals in group 4 (VAH) only 4 hours /day**

## Validation Study No. 3 “13-week infusion”

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**Strain:** Sprague Dawley

**Catheterization:** Catheter in the *Vena jugularis*

**Catheter attachment:** Instech Solomon Infusion Harness (Groups 1-3)  
Instech Solomon Vascular Access Harness (Groups 4-6)

**Breeding & operation:** Harlan Laboratories, B.V., The Netherlands

**Treatment period:** 13 weeks

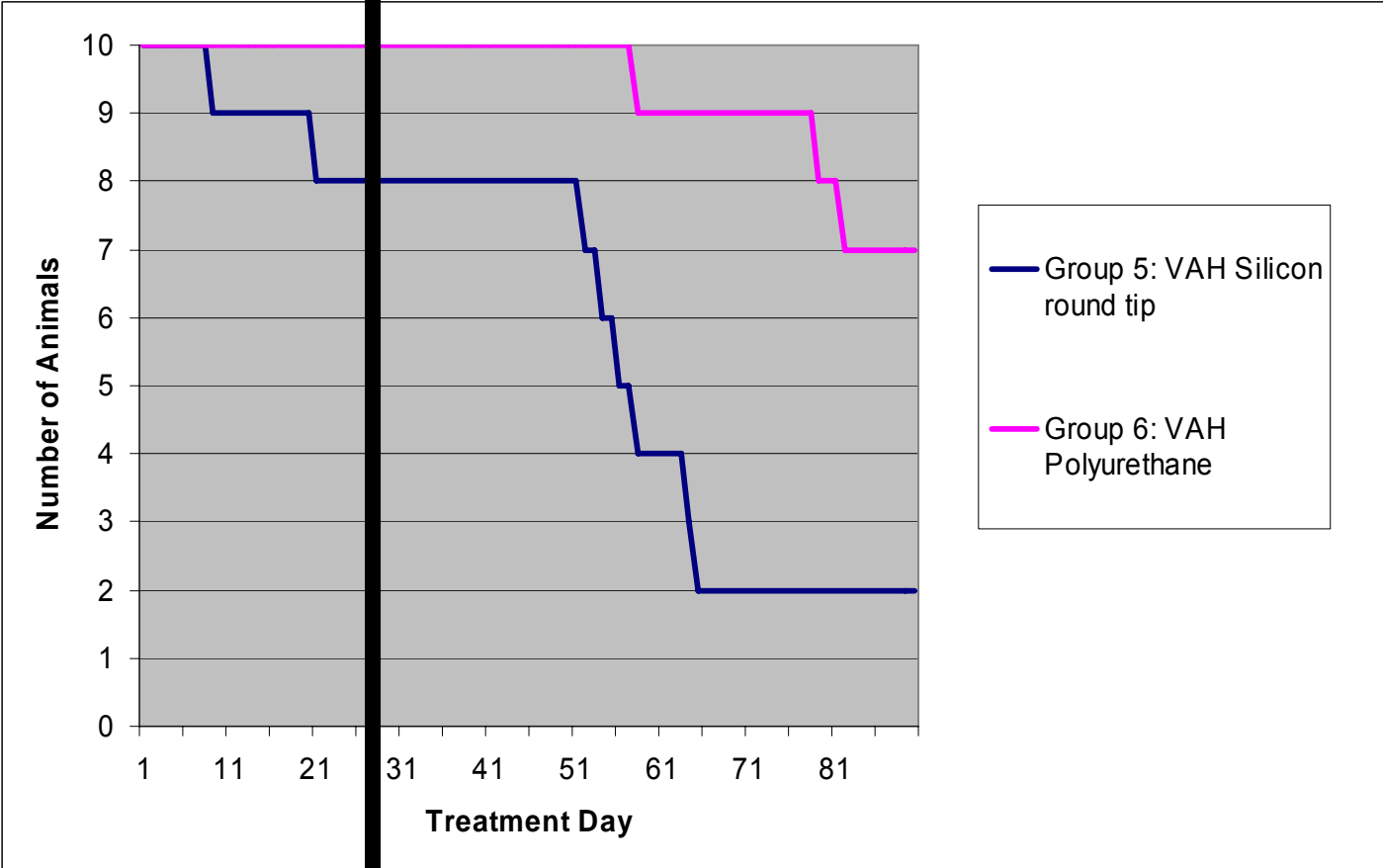
**Infusion:** 24 hours (groups 1, 2, 3, 5, 6), 4 hours (group 4)

	Substance	Dose Volume (ml/kg/h)	No. of Animals	Catheter	Infusion
Group 1	0.9% saline	3	10♂	polyurethane	24 hours
Group 2	0.9% saline	3	10♂	silicon	24 hours
Group 3	0.9% saline	3	10♂	silicon round tip	24 hours
Group 4	0.9% saline	3	10♂	silicon round tip	4 hours/day
Group 5	0.9% saline	3	5♂/5♀	silicon round tip	24 hours
Group 6	0.9% saline	3	5♂/5♀	polyurethane	24 hours

Additional Groups



# Validation Study No. 3 “13-week infusion”: Results



## Validation Study No. 3 “13-week infusion”: Results

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### Reason for the termination of animals (group 5)

- 1.) Catheter ripped out of vein – 2/10 animals
- 2.) Defective catheter – 2/10 animals
- 3.) Swellings – 3/10 animals

### Reason for the termination of animals (group 6)

Catheter ripped out of vein – 1/10 animals

Spontaneous death – 1/10 animals\*

General bad condition (Body weight loss, ruffled fur, etc.) – 1/10 animals

\* (Reason to be evaluated based on histopathology data)

## Validation Study No. 3 “13-week infusion”: Conclusions II

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Polyurethane catheter seems to be superior to the silicon catheter

4-week infusion studies can/should be performed with VAH without loss of animals related to the study design

## Overall Conclusions

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### After 7 years development of infusion studies we recommend:

1. Modified Dilab AccuSampler Cage with half of the lower part painted with a dark color, ventilation holes, removable plastic top, and a food rack with food pellets
2. Infusion pumps with back pressure sensor (superior to osmotic pumps)
3. Catheterized animals from Harlan Laboratories, B.V., The Netherlands (preferentially Sprague Dawley rats)
4. Jugular vein as catheter entry site
5. For studies with treatment up to 14 days: Dacron button (cutting the catheters for recovery period)
6. VAH with polyurethane catheters for studies longer than 14 days

## Outlook

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Use of Vascular Access Harness is the most suitable externalization technique for infusion studies

Use of Fresenius Kabi infusion filter with air separator to avoid accidental injections of air bubbles



## Outlook

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Can studies of 13 weeks be performed with VAH and a polyurethane catheter ?

Why is polyurethane better than silicon?

What can be done to minimize the animal losses towards the end of the treatment period? Why did so many animals rip out their catheters towards the end of the treatment period?

→ Evaluation of clinical diagnostic and histopathology data of the 13-week validation study are currently underway.

**Thank you !**

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Dilab (Sweden)

Instech Solomon (USA)

Harlan Laboratories Ltd.

Roland Sacher, Alexander Kohler, Andreas Otto, Ian Bunn

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