# Effect on Lung Permeation of Various Formulation Efforts Invested in the Inhaled Antibiotic Apramycin as Measured in the *Ex Vivo* Isolated, Perfused, and Ventilated Rat Lung

**Per Gerde**

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# Background

- The Isolated, ventilated and Perfused Lung of the rat (IPL), represents one versatile *ex vivo* model for use in preclinical drug development

- Integrated into the PreciseInhale® exposure platform, different aerosol formulations can be evaluated for lung PK and ventilation mechanics with a minimum of translational errors

- Four different formulations of the antibiotic Apramycin (Apr) was evaluated and compared for lung PK- and mechanics in the IPL system

# APRINHA - New Inhalation Formulations for Improved Delivery of Antimicrobials

Duration: 36 month  (Jan 2023 – Dec 2025)

Budget: 1 542 323 €

**Project Objectives**

Identify, characterize, and optimize a nanoparticle inhalation technology best suited for respiratory delivery of apramycin and other antimicrobials.

https://www.jpiamr.eu/projects/aprinha/

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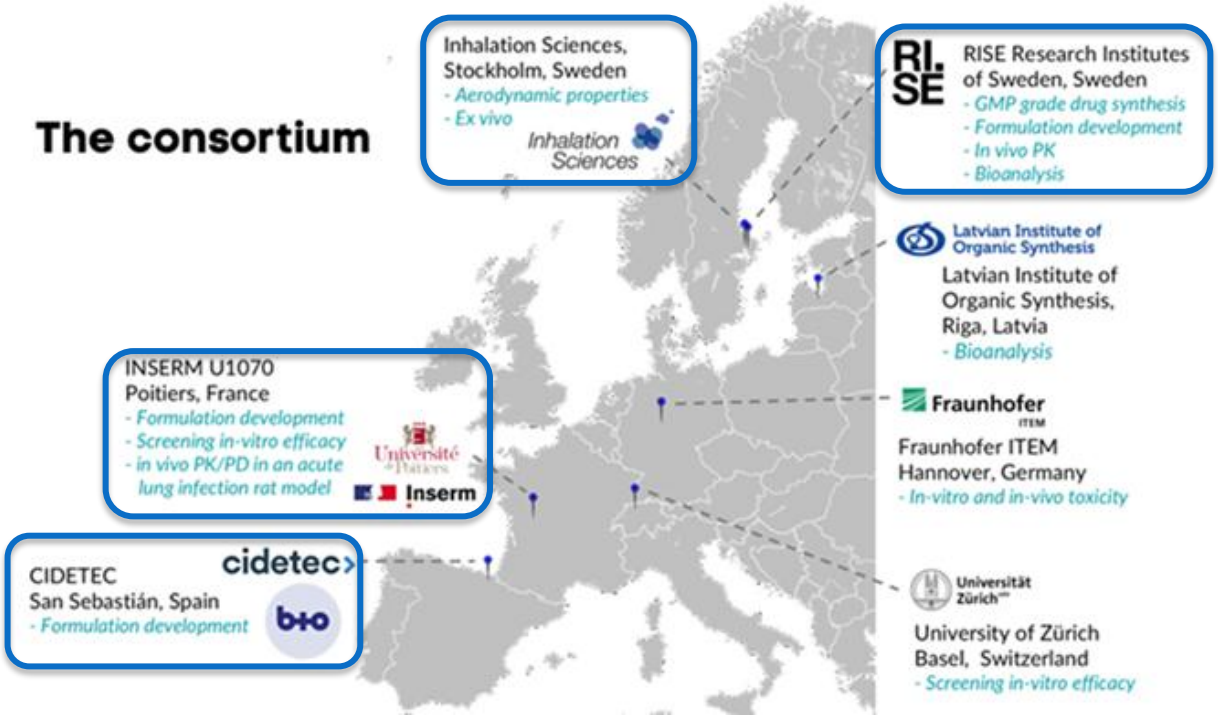
# APRINHA - a European multi-center study



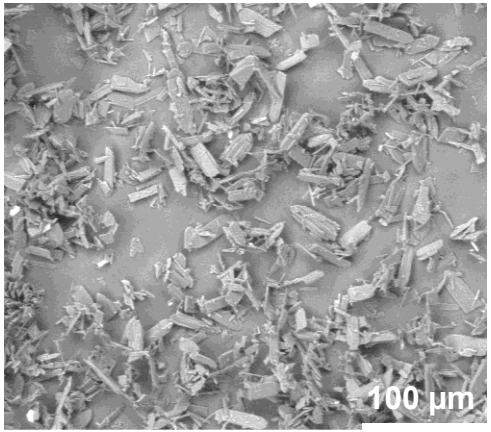
# Apramycin

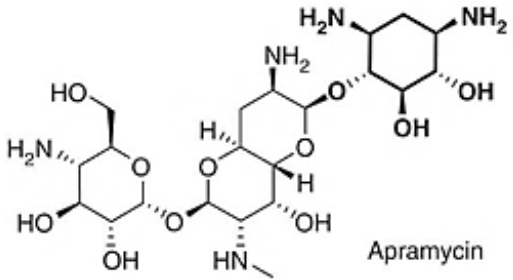
**Aminoglycoside antibiotic**

**Repurposed veterinary drug substance**

**Apramycin unique structure make it less susceptible to common aminoglycoside resistance mechanisms**

***DOI: 10.1021/jacs.9b11601***




**Physicochemical properties of Apramycin (free base)**

| Property | Value |
|---|---|
| **MW** | 539.58 g/mole |
| **Crystallinity** | Crystalline |
| **Melting point (Peak)** | 262 - 264 °C |
| **pKa[1]** | Basic: 7.7; 8.2; 8.4; 9.0; 9.6 |
| **Solubility in water[2]** | > 200 mg/mL below pH 8 |

[1]Predicted values Marvin sketch 5.12.1

[2]A commercial veterinarian product at 200 mg/mL is available in some European countries. Experimental work performed at RISE indicates a solubility of at least 500 mg/mL.

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# Inhalation formulation strategies for apramycin

## Formulation 1
Base line reference

**Ref-Apr**

- Apramycin solution (75 mg/mL, pH 5.5-6.0) for comparison of formulation performance

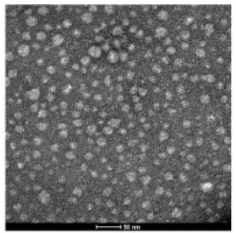
**Solution**

## Formulation 2
Dextran-based NP

**KuDa-Apr**

- Controlled release
- Biofilm penetration

**Suspension**

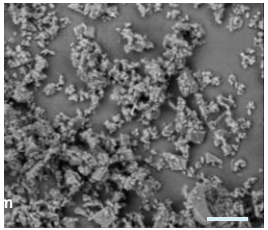
TEM. Dn = 13 ± 3 nm

## Formulation 3
Micronized apramycin

**DSPC-Apr**

- Decreased dissolution rate
- Low excipient to API

**Powder**

## Formulation 4
Apramycin: Ga complex

**Ga-Apr**

- Increased lung retention time
- Nanoparticle

**Powder**

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# The PreciseInhale® Inhalation Switchboard

## Aerosol sources

- Dry powder
- Clinical inhalers
- Nebulized solutions

## PreciseInhale®

## Exposure modules

**Dissolv*It*®** – *in vitro*
- Non-biological dissolution and absorption testing
- Physiology-like conditions with single pass perfusion
- $C_{max}$ and $T_{max}$ PK profile curves

**Xpose*ALI*®** – *in vitro*
- Lung-like exposures of lung cells in culture
- Permeability assessment

**Isolated Perfused Lung (IPL)** – *ex vivo*
- Lung absorption rate and retention data
- <15% SD in variability during repetetive dosing
- High-resolution data on lung PK- and mechanics

**Intratracheal inhalation in rodents** – *in vivo*
- Direct exposure lungs avoiding GI exposure
- Spontaneous breathing animals – physiological data

**Nose-Only inhalation in rodents** – *in vivo*
- Precision by design – one animal at the time
- Individual control of exposure data

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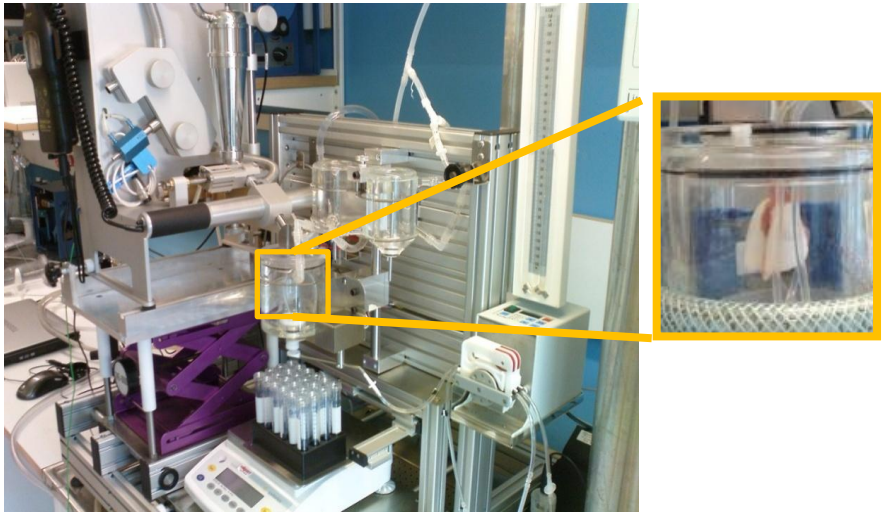
# Isolated, ventilated and perfused lung of the rat (IPL)



- Negative-pressure ventilated rat lung
- Aerosol exposure via a tracheal catheter
- Single-pass perfusion
- Two hour perfusate sampling time
- Monitoring of lung mechanics
- Over-all mass balance for dosing accuracy



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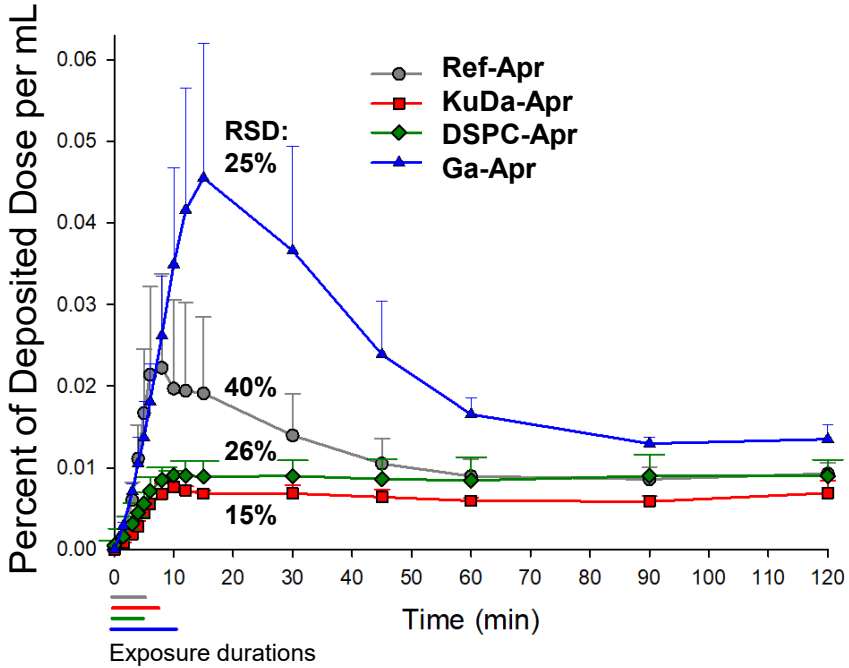
# Consistent Exposures with all Four Formulations of Apramycin

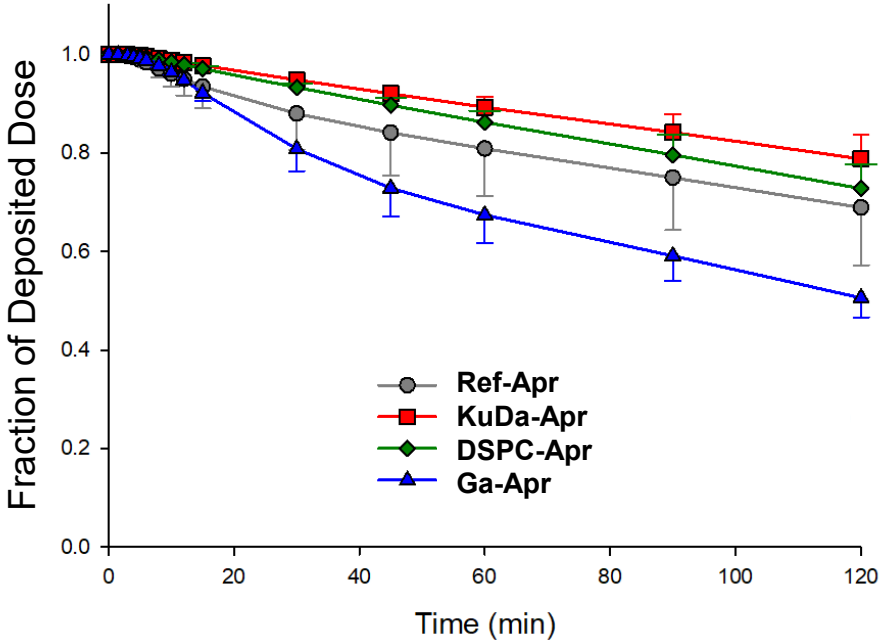
| Formulation | MMAD (μm) | GSD | Deposition (μg, ±SD) | Fraction of inhaled deposited (MPPD) Normalized regional deposition | | |
|---|---|---|---|---|---|---|
| | | | | Total of inhaled | Bronchial | Alveolar |
| **1. Ref-Apr Solution** | 2.8 | 1.9 | 133 ± 9% | 0.42 | 0.41 | 0.59 |
| **2. KuDa-Apr Suspension** | 3.6 | 2.2 | 151 ± 15% | 0.49 | 0.51 | 0.49 |
| **3. DSPC-Apr Powder** | 3.0 | 2.2 | 117 ± 11% | 0.43 | 0.46 | 0.54 |
| **4. Ga-Apr Powder** | 3.0 | 1.8 | 144 ± 12% | 0.45 | 0.43 | 0.57 |

# Distinctive Differences in Lung PK of Tested Formulations

## A. Dose-normalized concentration in perfusate




## B. Fraction of deposited retained in lung

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# Key Results of Lung PK for Studied Formulations

| Formulation | $C_{max}$ (ng/mL) | $T_{max}$ (min) | $t_{1/2}$ (h) | Fraction retained at 2h | Fractional distribution at 2h post exposure | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lung | ELF | Perfusate | |
| 1. Ref-Apr Solution | 33 ± 50% | 6.7 ± 17% | 4.1 ± 47% | 0.69 ± 17% | 0.56 ± 19% | 0.13 ± 20% | 0.31 ± 38% | Ref |
| 2. KuDa-Apr Suspension | 13 ± 11% | 9.3 ± 12% | 6.0 ± 23% | 0.79 ± 6% | 0.55 ± 6% | 0.24 ± 8% | 0.21 ± 23% | Slower |
| 3. DSPC-Apr Powder | 21 ± 20% | 7.0 ± 62% | 4.5 ± 24% | 0.73 ± 7% | 0.55 ± 11% | 0.14 ± 17% | 0.31 ± 22% | Slower |
| 4. Ga-Apr Powder | 71 ± 47% | 14 ± 12% | 1.9 ± 14% | 0.51 ± 8% | 0.34 ± 15% | 0.17 ± 35% | 0.49 ± 8% | Faster |

- Compared to Ref-Apr, KuDa-Apr and DSPC-Apr had an expected slower permeation in the lungs
- In contrast, Ga-Apr permeated the rat lung markedly faster than Ref-Apr

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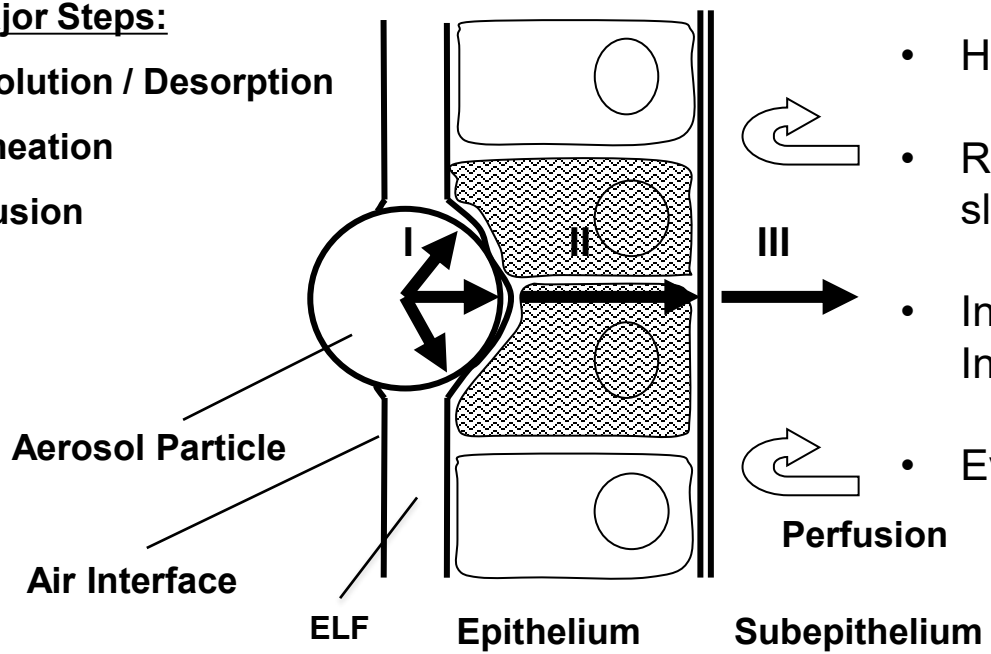
# The Pulmonary Absorption Process of a iBCS Class III Drug

**The Major Steps:**

**I Dissolution / Desorption**

**II Permeation**

**III Perfusion**




- High solubility, low permeability
- Rate control in step II; step I can only slow overall permeation
- Increase seen in Ga-Apr permeation! Increased barrier permeability?
- Evidence?

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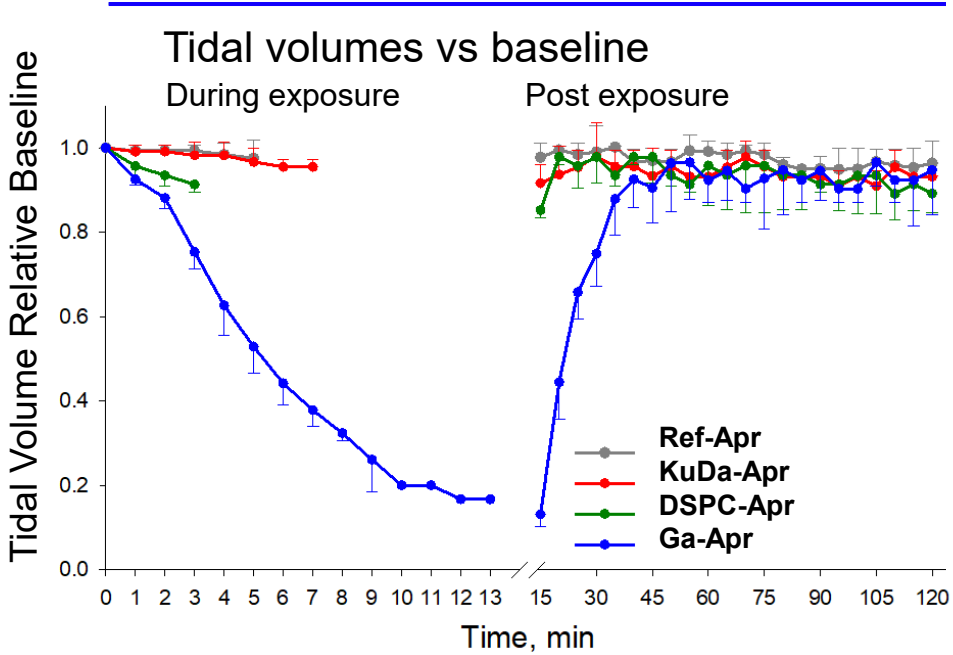
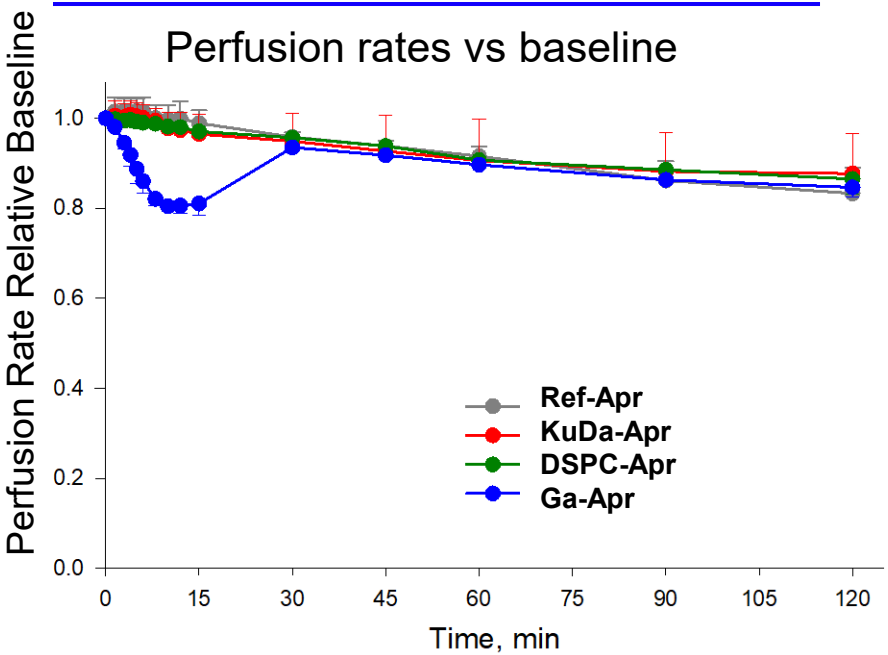
# Effect of Apramycin Formulation Exposures on Lung Ventilation and Perfusion Rates




- Ref-Apr, KuDa-Apr, and DSPC-Apr exposures had no noticable effect on lung physiology
- Ga-Apr caused a transient decrease in tidal volumes and perfusion rates for about 30-45 min
- No observable effect on lung physiology at the end of experiment

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# Conclusions

- For the iBCS Class III drug apramycin (high solubility and low permeability), it is possible to alter lung PK by using various engineered formulations
- Two formulations, KuDa-Apr and DPSC-Apr, slowed overall lung permeation compared to Ref-Apr
- One formulation, Ga-Apr, increased lung permeation compared to Ref-Apr, most likely by affecting the barrier function of the lung itself
- Low variability dosing with the PreciseInhale® system allow minimum number of animals to be used (n=3), still providing mechanistic understanding of lung PK (Reduce, Refine)

# Thank you!

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