13-week nose-only inhalation study of aerosolized propylene glycol (PG) in rats

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1. Introduction

PG in pharmaceutical formulations (excipient):

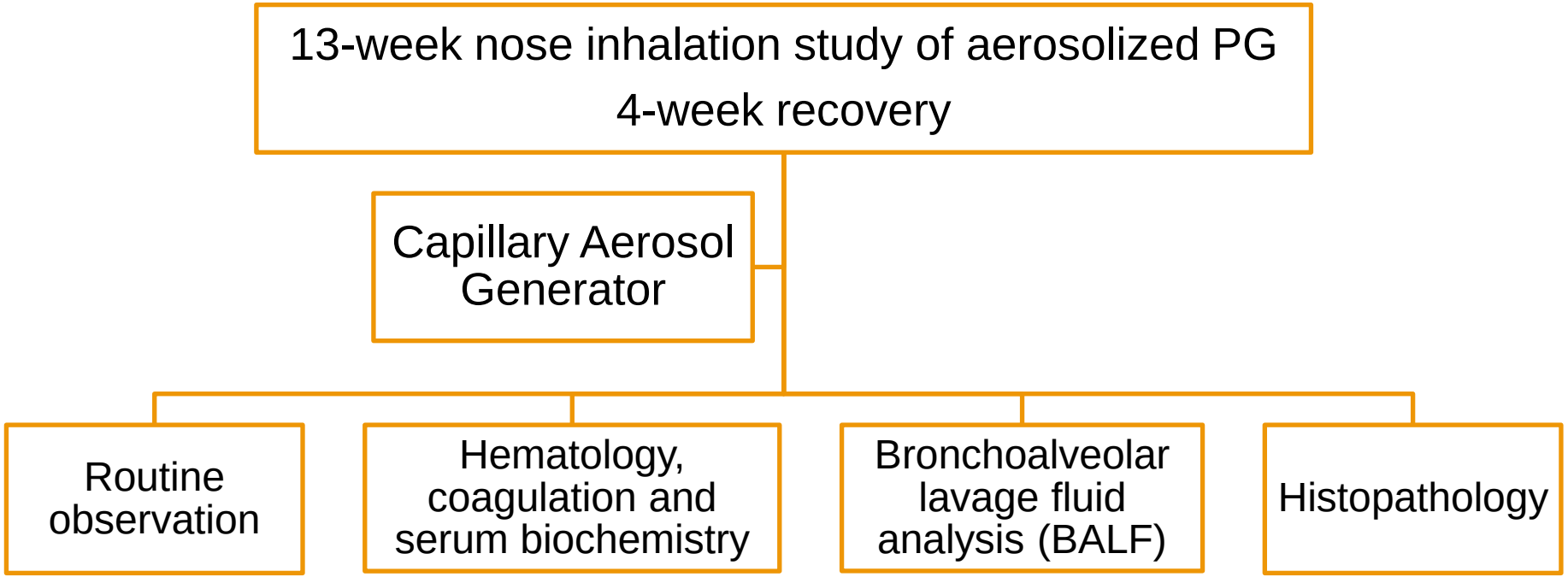
Asthma spray, inhalation solution

PG in e-cigarettes (up to 90%):

Chronic and high concentration PG aerosol exposure

The safety of its long-term inhalation exposure has not been adequately studied due to the limitation of atomization device

2. Experimental design



Animal and exposure dose design

All procedures involving animals were performed in an IACUC-SIFDC accredited. ***IACUC-SIFDC16129***

(*Institutional Animal Care and Use Committee-Shanghai Institute for Food and Drug Control*)

Animal	Group	Delivered Dose (mg/kg)	Exposure time (min/day)
Wistar Rats 8 Male and 8 Female	Control	0	78
	Low	100	6
	Mid	500	27
	High	1500	78

- **$DD = AC \times RMV \times D \times IF/BW$***
- **$RMV = 0.608 \times BW^{0.852}$**

DD: Delivered Dose (mg/kg)
AC: Aerosol Concentration(mg/L)
RMV: Respiratory Minute Volume(L/min)
D: Duration(min)
IF: Drug deposition coefficient
BW: Body Weight (kg)

*Alexander DJ, Collins CJ, Coombs DW, et al. (2008). Inhalation Toxicology 20(13): 1179-1189.

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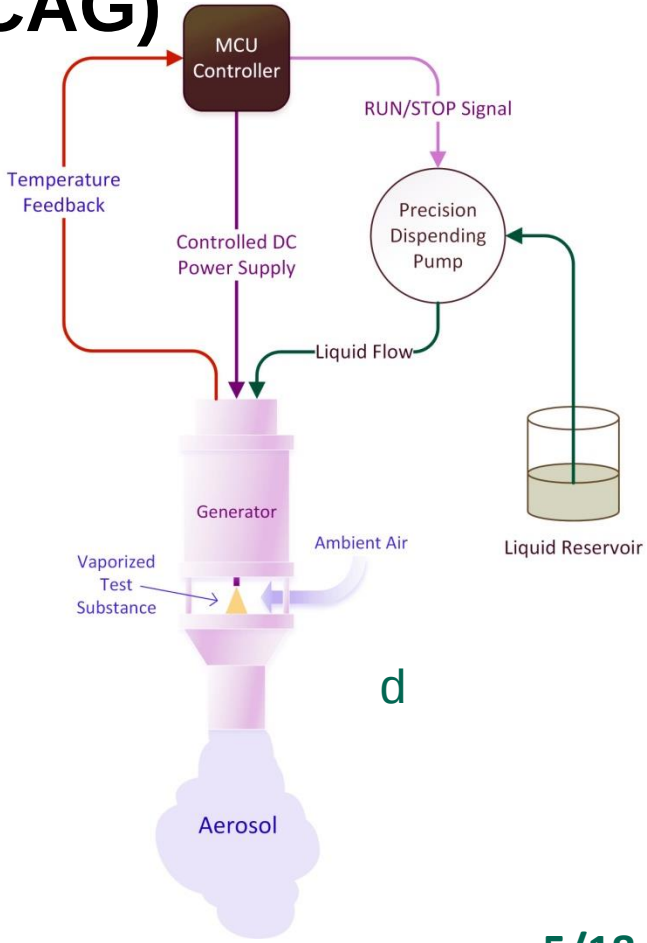
Capillary Aerosol generator (CAG)

- Aerosol Concentration :

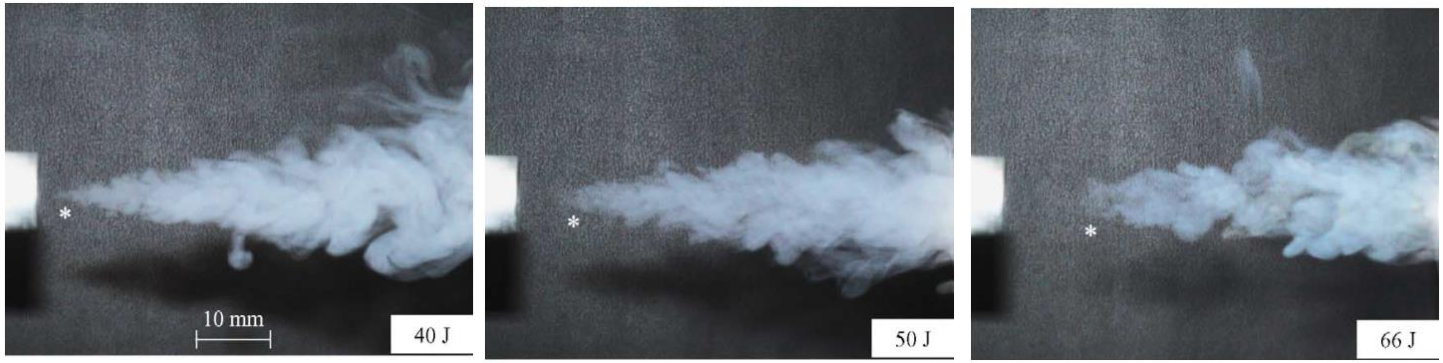
  Up to 40 mg/L

- Temperature:

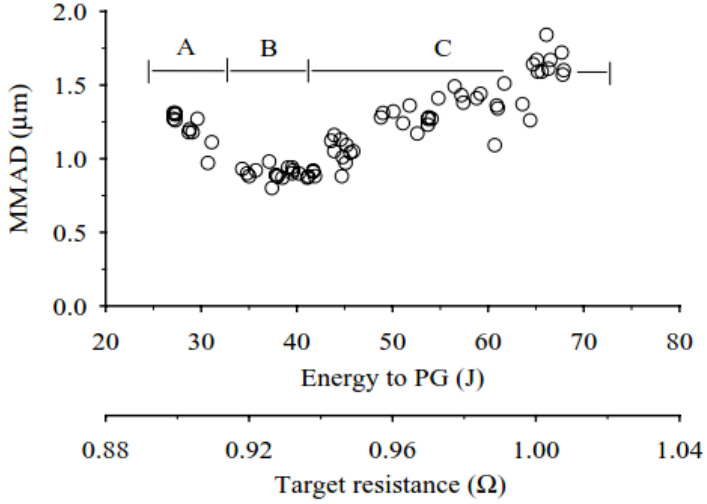
  300-400℃



PG aerosol produced by different energy



- Fine respirable particles in the range of 1-2 *μm*
- Reach the deep lung region and have quite high deposition



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Aerosol concentration and particle size data

Monitoring methods

- ☐Gravimetric
- ☐GC-FID
- ☐Next generation pharmaceutical impactor

Group	Exposure concentration(mg/L)	Mean MMAD(μm)	GSD	Sample (N=)
Low	29.19±1.85	1.59	1.34	10
Mid	29.40±0.69	1.56	1.37	10
High	28.65±1.72	1.57	1.34	10

MMAD: mass median aerodynamic diameter.
GSD: geometric standard deviation.

The aerosol particle is quite stable and fully respirable in rats

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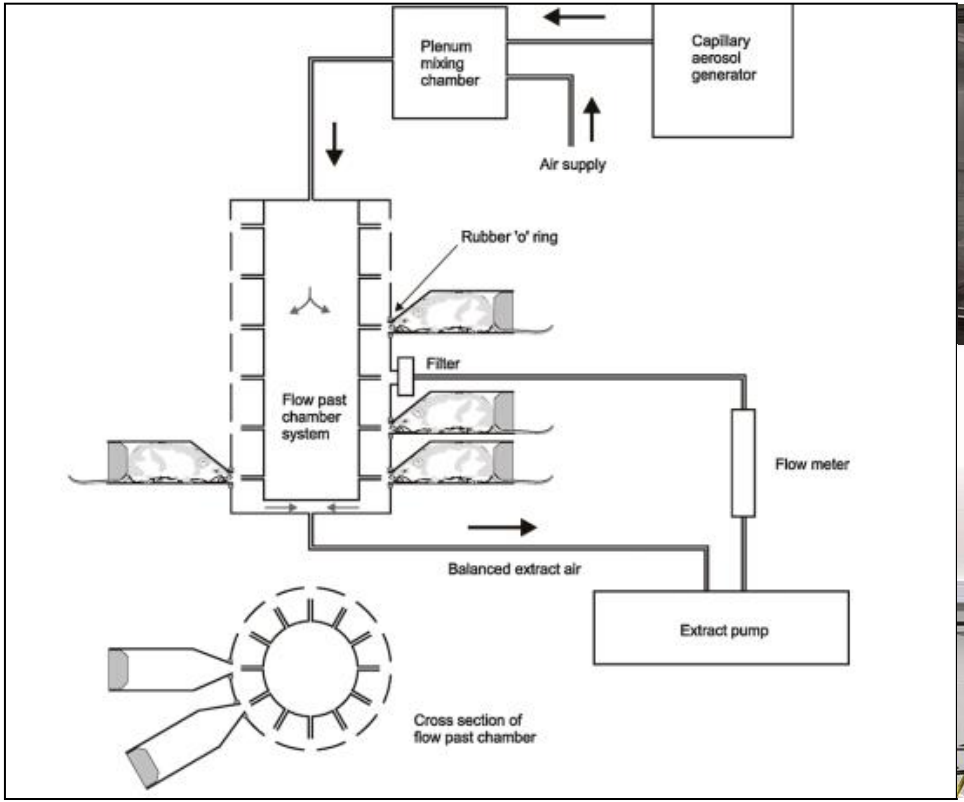
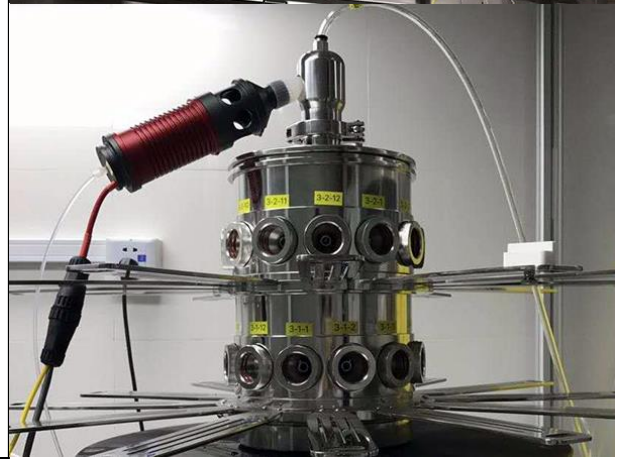
Nose-only inhalation exposure system





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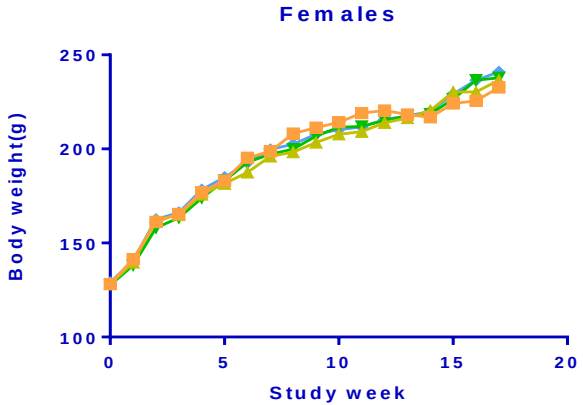
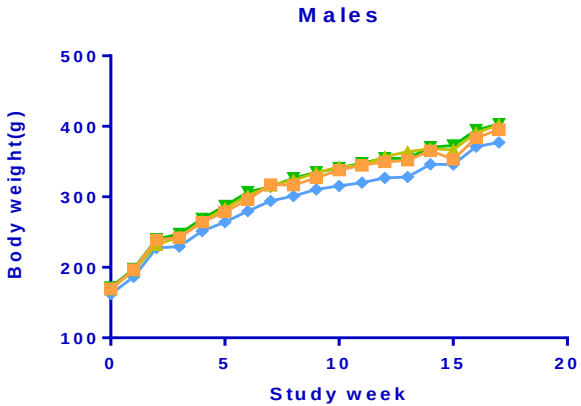
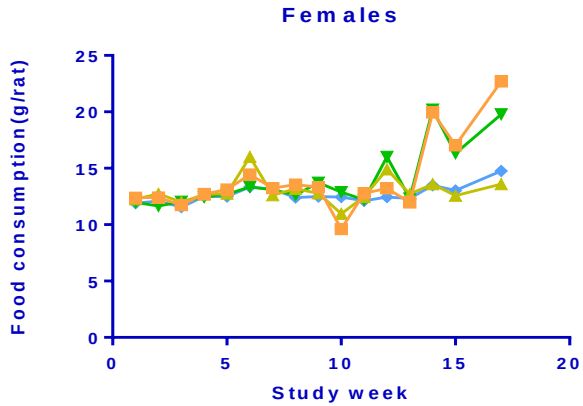
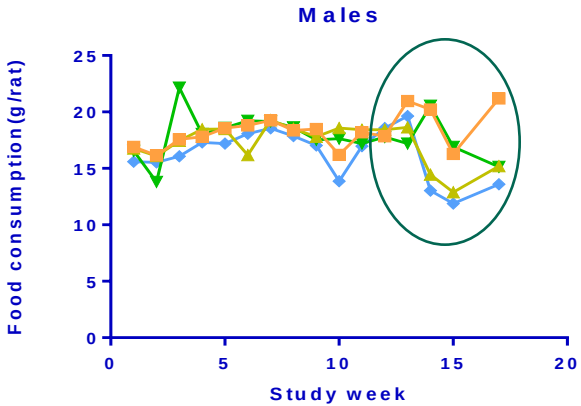
3. Results — *Body weights and food consumption*









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3. Results — *Clinical chemistry*

AST: aspartate aminotransferase
TP: total protein
TBIL: total bilirubin
BUN: blood urea nitrogen
CK: creatine kinase
Cl: chloride
K: potassium
Na: sodium

Parameter	13-week exposure		Recovery period	
	Male	Female	Male	Female
AST			↑M(47%)	
TP	↓H(3%)			
TBIL				↓H(33%)
BUN			↑M(13%)	↑M,H(18%)
CK			↑M(98%)	
Cl		↑L,H(2%)		
K		↓L,H(11%)		
Na	↓H(1%)			

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3. Results — *Hematology*

- No dose-dependent
- No sex difference
- No clear treatment-related changes in coagulation parameters
- All the observed differences were resolved at the recovery necropsy

Parameter	13-week exposure	
	Male	Female
WBC		↓L(40%)
RBC	↓M,H(7%)	
HGB	↓H(6%)	
HCT	↓H(7%)	
Lymph		↓L(41%)

WBC: white blood cell count;
RBC: red blood cell count;
HGB: hemoglobin;
HCT: hematocrit;
Lymph: lymphocyte

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3. Results — *Bronchoalveolar Lavage Fluid*

Parameter	Units	BALF after 13-week exposure in male rats			
		Control	Low	Mid	High
Total count	$\times 10^9$	1.51±0.54	1.38±0.37	1.52±0.45	1.45±0.26
Alveolar macrophages	%	95.58±0.89	94.90±1.33	**85.90±1.16**	**80.75±0.26**
Neutrophils	%	3.58±0.89	3.93±1.33	**10.48±0.71**	**15.43±0.09**
Lymphocytes	%	0.75±0.30	1.01±0.30	**3.55±0.70**	**3.63±0.63**
ALP	U/L	62.50±10.34	91.50±39.03	68.25±28.18	50.75±11.44
LDH	U/L	196.55±36.88	214.65±84.87	176.20±82.47	168.90±78.66
TP	μg/ml	466.27±133.95	548.12±194.08	461.93±129.74	498.52±115.11

ALP: alkaline phosphatase; **LDH:** Lactate dyhydrogenase; **TP:** total protein

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3. Results — *Bronchoalveolar Lavage Fluid*

Parameter	Units	BALF after 13-week exposure in female rats			
		Control	Low	Mid	High
Total count	$\times 10^9$	1.08±0.30	1.08±0.25	1.55±0.50	1.40±0.33
Alveolar macrophages	%	94.30±1.22	94.00±0.79	**83.63±2.21**	**79.15±0.47**
Neutrophils	%	3.95±0.70	4.93±1.43	**15.20±1.48**	**19.28±4.73**
Lymphocytes	%	1.55± 0.70	1.03±0.06	1.13±0.12	1.37±0.72
ALP	U/L	108.25±88.50	73.75±23.00	112.75±93.18	72.50±22.52
LDH	U/L	301.28±184.91	224.75±49.01	287.50±194.35	193.23±67.33
TP	μg/ml	576.02±196.81	455.73±48.70	523.94±224.68	592.16±245.59

ALP: alkaline phosphatase; **LDH:** Lactate dyhydrogenase; **TP:** total protein

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3. Results — *Bronchoalveolar Lavage Fluid*

BALF analysis after recovery period

Parameter	Male Group			Female Group		
	Control	**Mid**	**High**	Control	**Mid**	**High**
Total count	1.13±0.29	1.17±0.45	1.18±0.53	1.21±0.42	1.17±0.70	0.68±0.35
Alveolar macrophages	99.00±1.10	**98.90±0.91**	**98.75±8.19**	98.00±2.10	**97.75±1.91**	**97.55±3.23**
Neutrophils	0.58±0.88	**0.48±0.92**	**0.43±0.43**	0.90±0.67	**0.98 ±0.35**	**1.15±0.78**
Lymphocytes	0.40±0.80	**0.55±0.81**	**0.59±0.74**	0.88±0.49	0.97±0.97	0.99±0.72

All abnormities in BALF cytology completely resolved during recovery

3. Results — *Histopathology*

- No macroscopic changes were observed
- All animals are solitary pathological changes

Incidences of histopathological findings

Parameter	13-week				Recovery			
	Control	High	Mid	Low	Control	High	Mid	Low
Lung	4/8	6/8	/	/	3/8	3/8	/	/
Pancreas	0/8	2/8	/	/	0/8	0/8	/	/
Liver	0/8	0/8	/	/	1/8	1/8	/	/
Kidney	0/8	0/8	/	/	2/8	0/8	/	/
Vagina	0/4	1/4	/	/	0/4	0/4	/	/
Breast	1/4	0/4	/	/	0/4	0/4	/	/
Harderian Gland	6/8	4/8	5/8	4/8	5/8	5/8	5/8	3/8

4. Discussion

- Body weight and food consumption in male rats tended to decrease

- The low-dose group of body gains for female showed a significant reduction at 8th week compared with control group

- Inhalation of PG aerosol has an inhibitory effect on the appetite of animals

4. Discussion

- BALF analysis showed lung inflammatory responses in male and female at 13-week.

- Histopathological findings including the lungs did not indicate degree and incidences of lung injury.

- All abnormities in lung cytology completely resolved during recovery.

5. Conclusion

VS

Exposure concentration 1 mg/L

Duration: 60 min

Body Weight: 60 kg

IF: 10%

NOEL(no observed effect level) for rat

100mg/kg

Maximum exposure dose per day

2mg/kg

PG could be an appropriate solvent without significant toxic effect for long-term exposure

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THANK YOU

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