

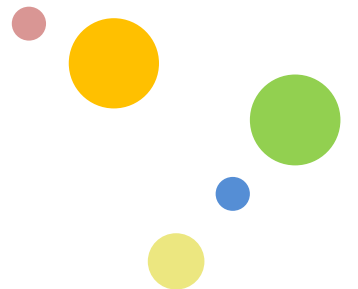


# Comparison of *in vitro* toxicity of heated tobacco products and combustible cigarette.

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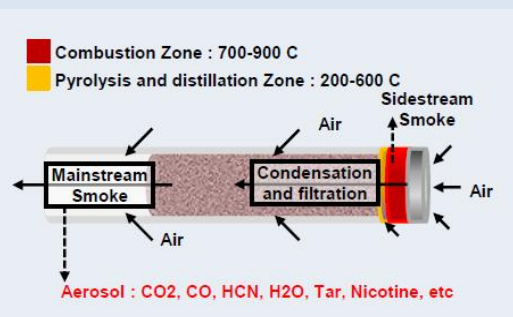
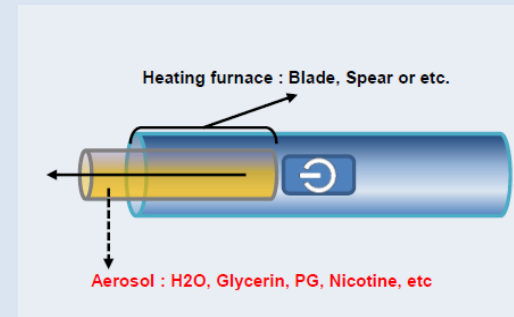


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- Aerosol chemistry
- *In vitro* toxicity
- Conclusions

## ▶ Heated tobacco products(HTPs)

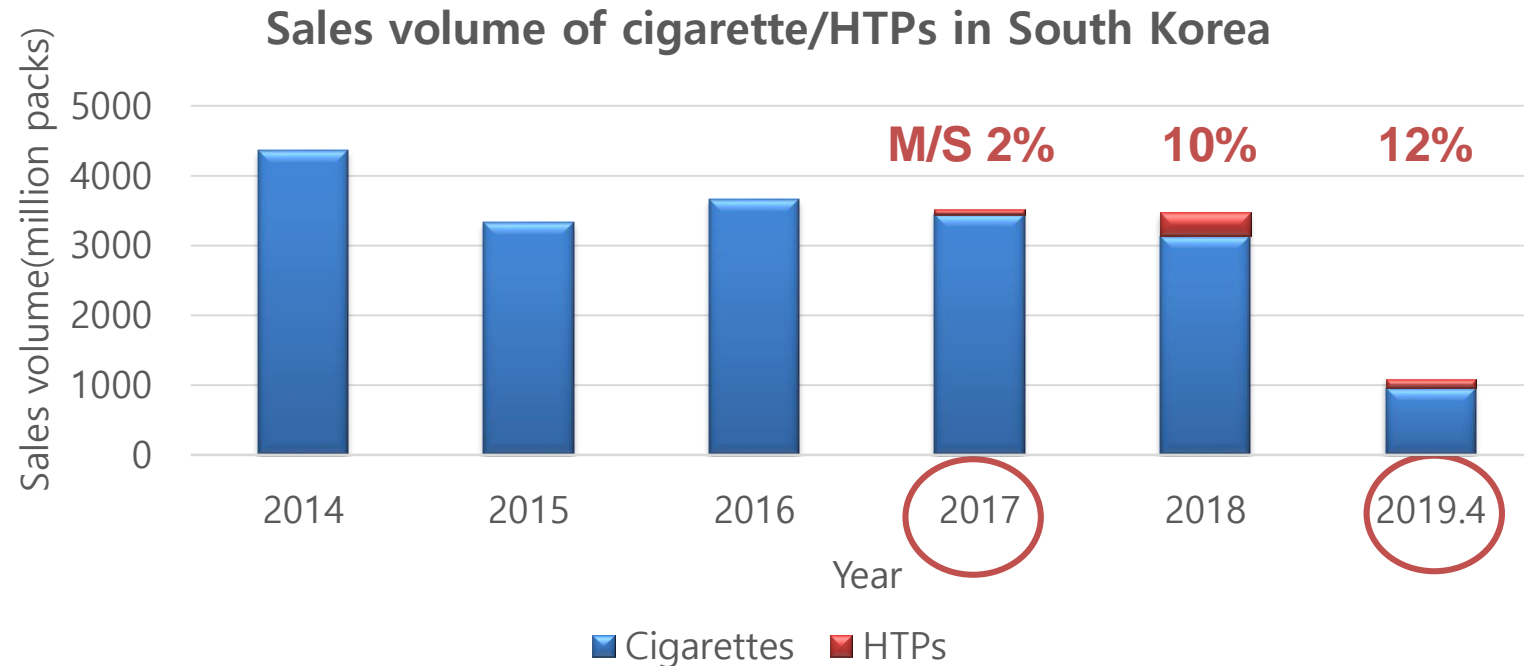
- Conventional products VS Heated tobacco products

|              | Conventional cigarette  | Heated tobacco products  |
|--------------|---|--|
|              |  <p> <span style="color: red;">■</span> Combustion Zone : 700-900 C<br/> <span style="color: orange;">■</span> Pyrolysis and distillation Zone : 200-600 C         </p> <p>           Mainstream Smoke, Condensation and filtration, Sidestream Smoke, Air, Aerosol : CO<sub>2</sub>, CO, HCN, H<sub>2</sub>O, Tar, Nicotine, etc         </p> |  <p>           Heating furnace : Blade, Spear or etc.<br/>           Aerosol : H<sub>2</sub>O, Glycerin, PG, Nicotine, etc         </p> |
| Tobacco      | Blended tobacco   | Specially devised reconstituted tobacco  |
| Heating type | Combustion (<900°C)   | Electrically controlled heating system (<350°C)  |

- HTPs are known to be less harmful than conventional cigarette.
- HTPs met the needs of consumers concerned about sidestream smoke, tobacco odor and health risks of conventional cigarette.
- HTPs are becoming popular not only for smokers looking for less harmful products, but also for smokers who want to quit smoking.

## ▶ Heated tobacco products market

- South Korea is world's No.2 market(2018)
- M/S of HTPs has been to 12% this year.






| Products                         | 3R4F  | Type A   | Type B  |
|----------------------------------|---|--|---|
| Characteristics                  | Kentucky reference cigarette<br><br>combustible tobacco product | Heated tobacco product<br><br>produces aerosols by directly heating tobacco stick with an internal heat source | Heated tobacco product (a hybrid of a vapor and HTP)<br><br>- equipped with an additional liquid cartridge containing humectants<br>- aerosol from the e-liquid cartridge passes through the stick tobacco to deliver the flavor & nicotine |
| Tobacco heating temperature (°C) | <900  | 300~350  | 160   |
| Description of operation         | Combustion  | Distillation, Condensation   | Distillation, Condensation  |

## ▶ Harmful substances in tobacco smoke/aerosol

- A mixture of thousands of chemicals, of which about 100 constituents are known to be associated with tobacco-related diseases.(Reinskje Talhout et al., 2011)
- List of hazardous substances proposed by each agency.



|                  |  |
|------------------|--|
| PMI 58           | •Ethylene oxide, Styrene, Lead, Acetamide....            |
| Hoffman 44       | •Tar, Nicotine, NO, HCN, Catechol, Phenol....            |
| FDA ENDS PMTA 33 | •Cadmium, Benzyl acetate, Diacetyl, Propylene glycol.... |
| FDA HPHC 18      | •Acetaldehyde, Acrylonitrile, Ammonia, 1,3-Butadiene...  |
| IARC 15          | •Formaldehyde, NNK, NNN, Vinyl chloride...               |
| WHO 9            | •Carbon monoxide, Benzo[a]pyrene, Acrolein...            |
|                  |  |
|                  |  |
|                  |  |

- PMI 58 list contains most of these constituents comprehensively.
- It was selected as the basis for chemical analysis of tobacco smoke/aerosol.

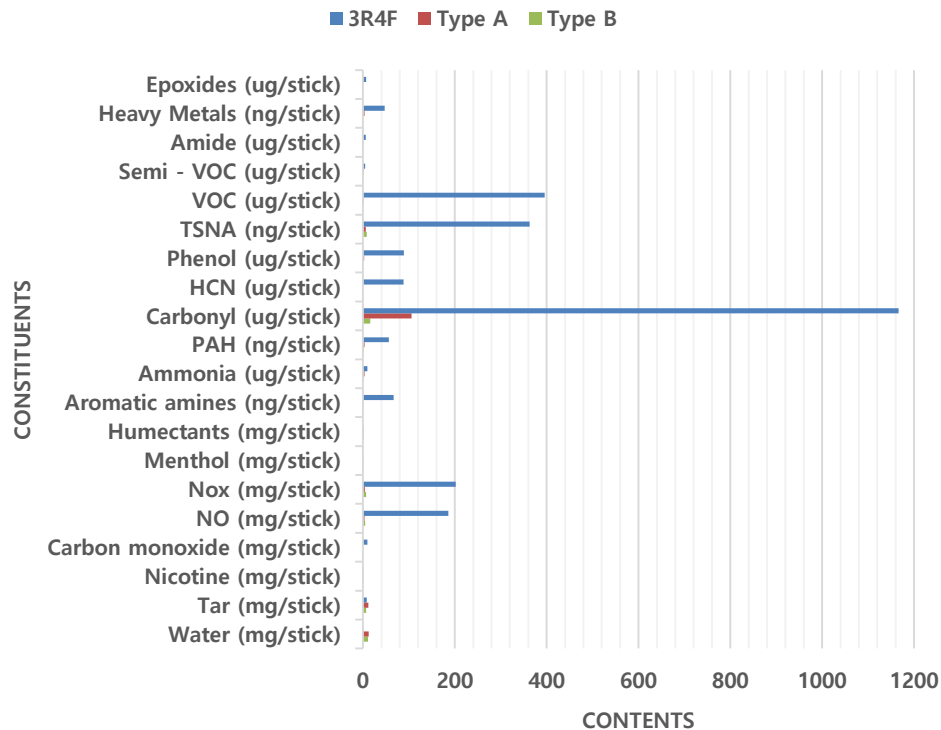
- ▶ Testing institution : Labstat International Inc.
- ▶ Smoking regime

|     | Puff interval (sec) | Puff volume (mL) | Puff duration (sec) | Vent blocking (%) | Smoked Puff Number (HTP) |
|-----|---------------------|------------------|---------------------|-------------------|--------------------------|
| ISO | 60                  | 35               | 2                   | 0                 | 4                        |
| HC  | 30                  | 55               | 2                   | 100               | 8                        |

- ▶ Analytical constituents

Aromatic amines(5)+PAHs(4)+Carbonyls(8)+Phenols(6)+TSNAs(4)+VOCs(6)  
 +Semi-VOCs(4)+Amides(2)+Heavy metals(7)+Epoxides(2)+NOx(2)  
 +Humectants(2)+HCN+Ammonia+Menthol+CO+Nicotine+Tar+Water

## Results of aerosol analysis (ISO)

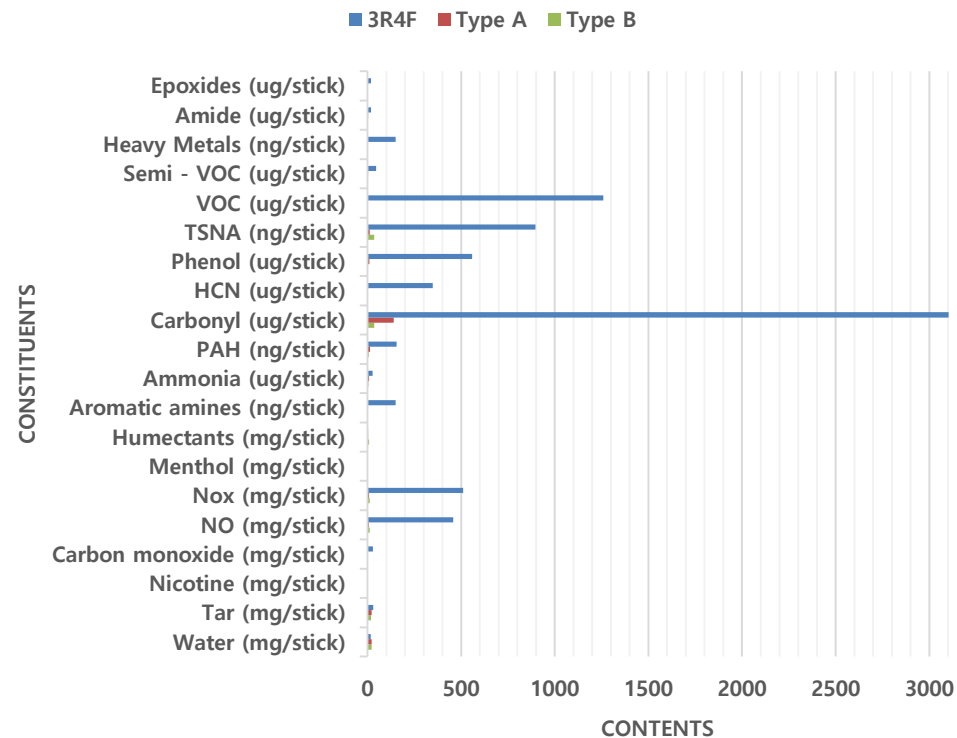


| Relative value(%) | 3R4F   | Type A | Type B |
|-------------------|--------|--------|--------|
| Tar               | 100.00 | 143.03 | 82.84  |
| Nicotine          | 100.00 | 43.06  | 8.47   |
| Carbon monoxide   | 100.00 | <LOQ   | <LOQ   |
| Aromatic amines   | 100.00 | <LOQ   | <LOQ   |
| Ammonia           | 100.00 | 35.60  | 12.96  |
| PAH               | 100.00 | <LOQ   | <LOQ   |
| Carbonyl          | 100.00 | 11.45  | 1.48   |
| HCN               | 100.00 | 9.08   | 1.37   |
| Phenol            | 100.00 | <LOD   | <LOQ   |
| TSNA              | 100.00 | 1.59   | 2.21   |
| VOC               | 100.00 | 0.23   | <LOQ   |
| Semi-VOC          | 100.00 | 24.35  | 6.85   |
| Amide             | 100.00 | 7.52   | <LOQ   |
| Epoxides          | 100.00 | <LOQ   | <LOQ   |
| Heavy Metals      | 100.00 | 7.52   | <LOQ   |

- Most of constituents have been significantly reduced for HTPs compared with 3R4F.
- Average aerosol emissions of both HTPs were more than 86% lower than that of 3R4F.
- Emissions of type B was 42% less than that of type A.



## Results of aerosol analysis (HC)

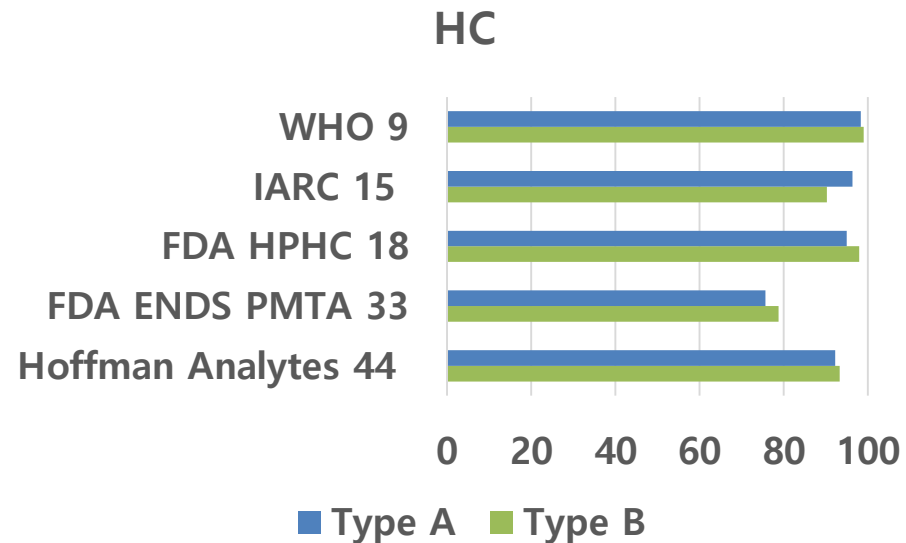
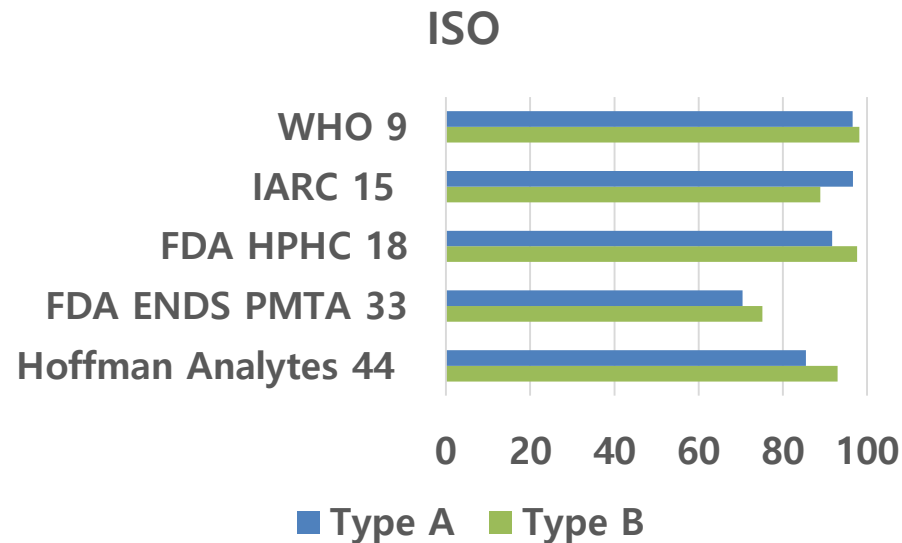


| Relative value(%) | 3R4F   | Type A | Type B |
|-------------------|--------|--------|--------|
| Tar               | 100.00 | 73.58  | 59.20  |
| Nicotine          | 100.00 | 42.05  | 9.23   |
| Carbon monoxide   | 100.00 | <LOQ   | <LOQ   |
| Aromatic amines   | 100.00 | 0.61   | 0.16   |
| Ammonia           | 100.00 | 29.09  | 14.89  |
| PAH               | 100.00 | 8.75   | 3.98   |
| Carbonyl          | 100.00 | 4.50   | 1.11   |
| HCN               | 100.00 | <LOQ   | <LOQ   |
| Phenol            | 100.00 | 1.87   | 0.00   |
| TSNA              | 100.00 | 1.36   | 3.89   |
| VOC               | 100.00 | 0.14   | 0.00   |
| Semi-VOC          | 100.00 | 5.46   | 0.60   |
| Amide             | 100.00 | 13.31  | 1.48   |
| Epoxides          | 100.00 | 0.67   | 0.00   |
| Heavy Metals      | 100.00 | 0.95   | 0.00   |

- Average aerosol emissions of both HTPs was more than 92% lower than that of 3R4F.
- Emissions of type B was 28% less than that of type A.

## Summary of aerosol chemistry

- Emission of aerosol constituents was significantly reduced for HTPs compared with 3R4F.
- Average % reduction in type B was slightly higher than in type A.
- Harmful constituents presented by other regulatory agencies also decreased by 70-99% in HTPs.



\* Average % reduction : average of individual reduction rates by analytical constituents

## ▶ Sample preparation

### Smoke generation (for toxicity test)

- In order to assess the toxicity, aerosol constituents should be collected as much as possible.
  - Rotary smoking machine under HC smoking regime
  - HTPs : fixed to 9 puff

### Preparation of test material

- **TPM(Total particulate matter)**
  - trapped on Cambridge filters, weighed collection to determine the total weight TPM and extracted continuously with DMSO.
- **GVP(Gas vapor phase)**
  - the smoke/aerosol, which passed through Cambridge filter, was bubbled through ice-cold PBS.

### Limitations

- In order to evaluate heated tobacco products in the same way as conventional cigarette evaluation, it took much more time and labor and was inefficient.
- There is no standard extraction method for heated tobacco products.

## ▶ Genotoxicity test

### - Bacterial reverse mutation test (Ames test)

-*Salmonella typhimurium*

-known to cause direct mutations in DNA and is also highly associated with carcinogenesis.

-Based on OECD Guideline

-TA98, TA100, TA102, TA1535, TA1537 ± S9 mix

-(TPM) Treatment up to 5000 µg/plate for HTPs

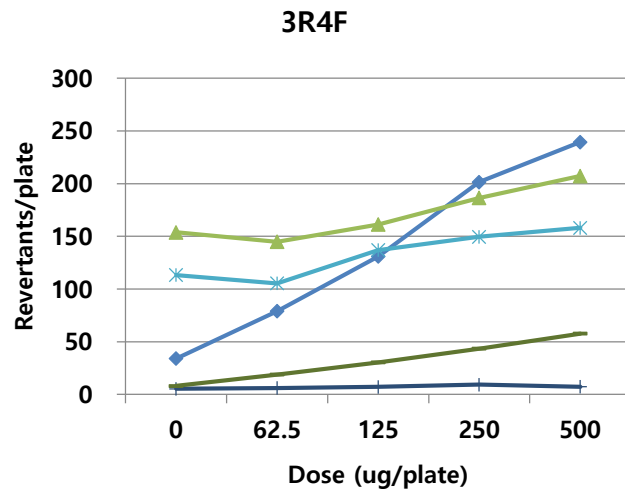
### - Acceptance criteria for positive determination

#### • Evaluation of mutagenic response

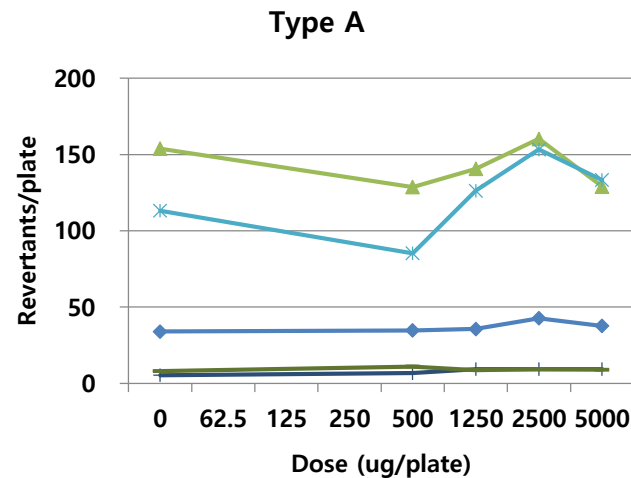
- ① a concentration-related increase in revertant numbers is observed over the concentration range tested
  - ② a statistically significant increase in the mean revertant number is observed for at least one concentration compared to solvent control using the Dunnett's test ( $p < 0.01$ ).
  - ③ Revertant counts outside the distribution of the historical negative (solvent) control data
- **Mutagenic in the replicate assay if all of the above criteria are met.**

## Results of Ames test (+S9 Mix)

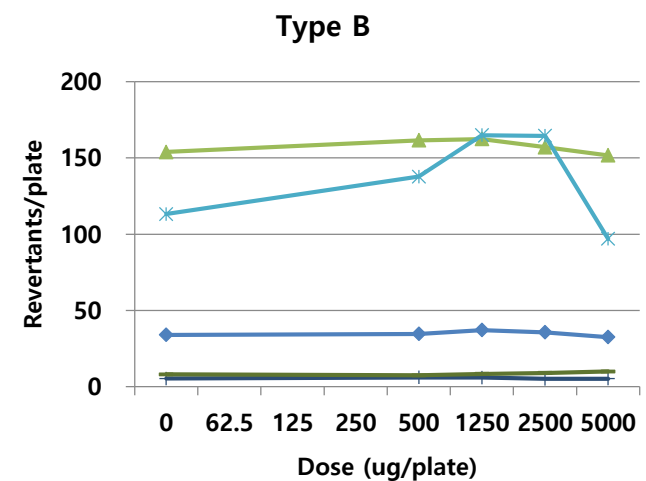
- 3R4F was positive in TA98, TA100 and TA1537 strains.
- HTPs were negative in all strains tested.



◆ TA98+S9    ▲ TA100+S9    \* TA102+S9  
 + TA1535+S9    ■ TA1537+S9



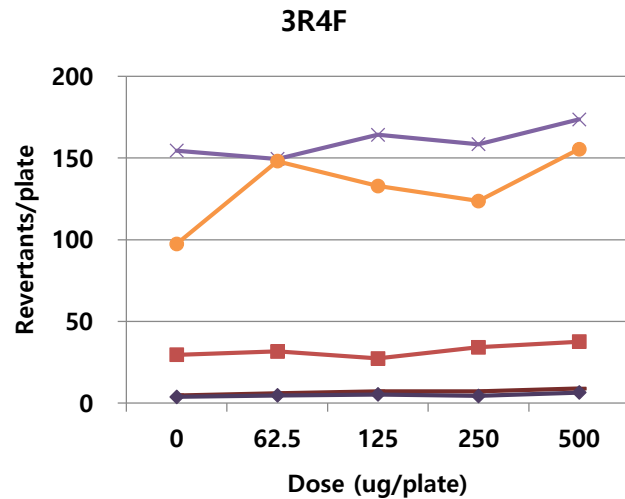
◆ TA98+S9    ▲ TA100+S9    \* TA102+S9  
 + TA1535+S9    ■ TA1537+S9



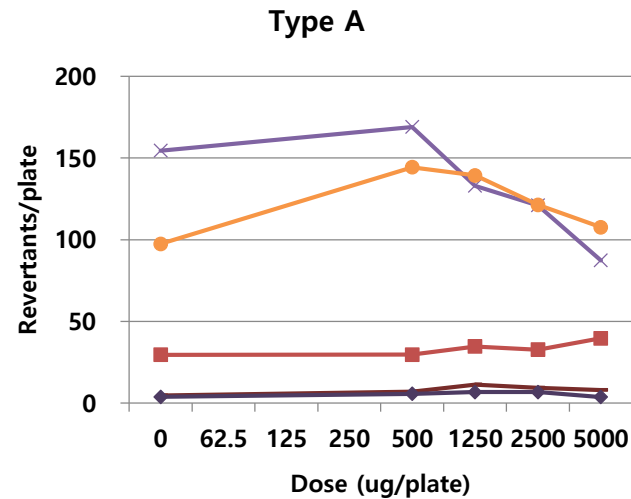
◆ TA98+S9    ▲ TA100+S9    \* TA102+S9  
 + TA1535+S9    ■ TA1537+S9

## Results of Ames test (-S9 Mix)

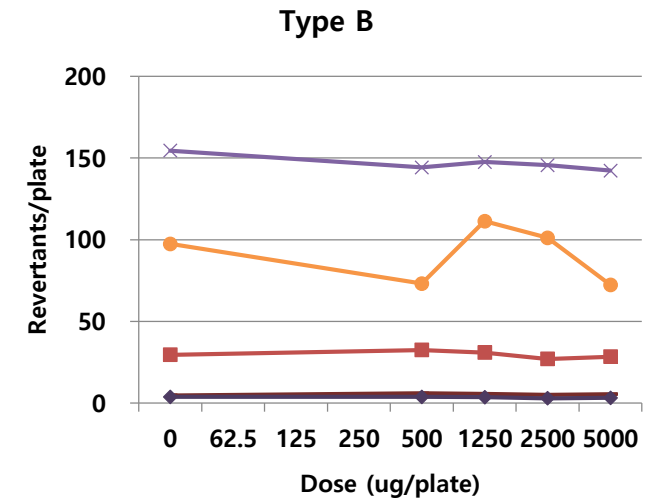
- Both 3R4F and HTPs showed negative mutagenicity.



■ TA98-S9    × TA100-S9    ● TA102-S9  
 — TA1535-S9    ◆ TA1537-S9



■ TA98-S9    × TA100-S9    ● TA102-S9  
 — TA1535-S9    ◆ TA1537-S9



■ TA98-S9    × TA100-S9    ● TA102-S9  
 — TA1535-S9    ◆ TA1537-S9

## ▶ Summary of Ames test

### - Comparison of mutagenicity for HTPs and 3R4F

+ : Positive / - : Negative

| Test products | TA98 |     | TA100 |     | TA102 |     | TA1535 |     | TA1537 |     |
|---------------|------|-----|-------|-----|-------|-----|--------|-----|--------|-----|
|               | +S9  | -S9 | +S9   | -S9 | +S9   | -S9 | +S9    | -S9 | +S9    | -S9 |
| 3R4F          | +    | -   | +     | -   | -     | -   | -      | -   | +      | -   |
| Type A        | -    | -   | -     | -   | -     | -   | -      | -   | -      | -   |
| Type B        | -    | -   | -     | -   | -     | -   | -      | -   | -      | -   |

## ▶ Cytotoxicity test

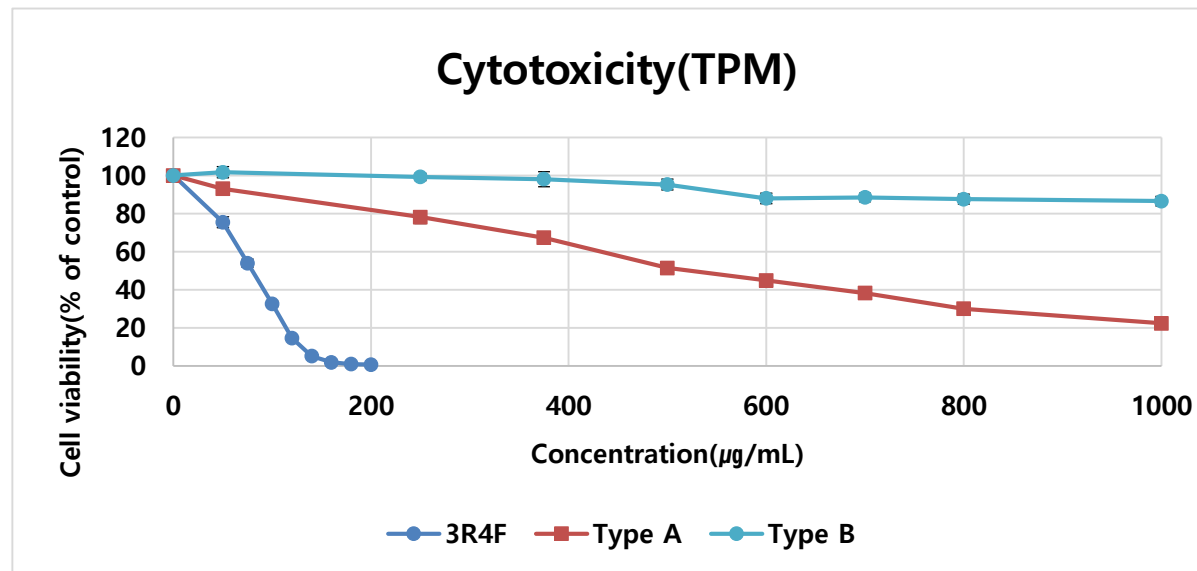
### - NRU(Neutral red uptake) assay

- Mammalian cell (CHO cell)
- widely used in the toxicity evaluation of tobacco smoke/aerosol
- $IC_{50}$  : concentration showing 50 % cytotoxicity
- (TPM) Treatment up to 1000  $\mu\text{g}/\text{mL}$ (2% DMSO)
- (GVP) Treatment up to 5000  $\mu\text{g}$  equivalent TPM/ $\text{mL}$ (10% PBS)



## ▶ Results of NRU assay (TPM)

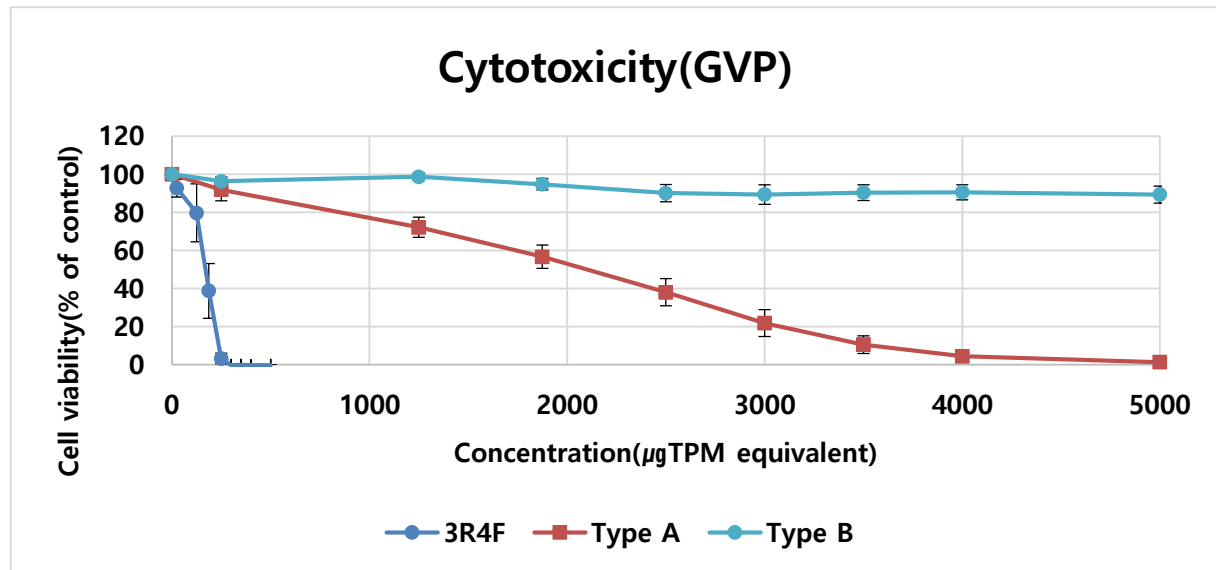
- Comparison of cytotoxicity for TPM of HTPs and 3R4F



- 3R4F showed dose-dependent decreases in cell viability.
- Type A and type B showed a survival rate of about 33% and 90%, respectively at the highest concentrations and type B did not show any measurable  $IC_{50}$ .

## ▶ Results of NRU assay (GVP)

- Comparison of cytotoxicity for GVP of HTPs and 3R4F



- 3R4F and type A showed dose-dependent decreases in cell viability.
- Type B showed a survival rate of about 90% at the highest concentrations and did not show any measurable  $IC_{50}$ .

## ► Summary of NRU assay

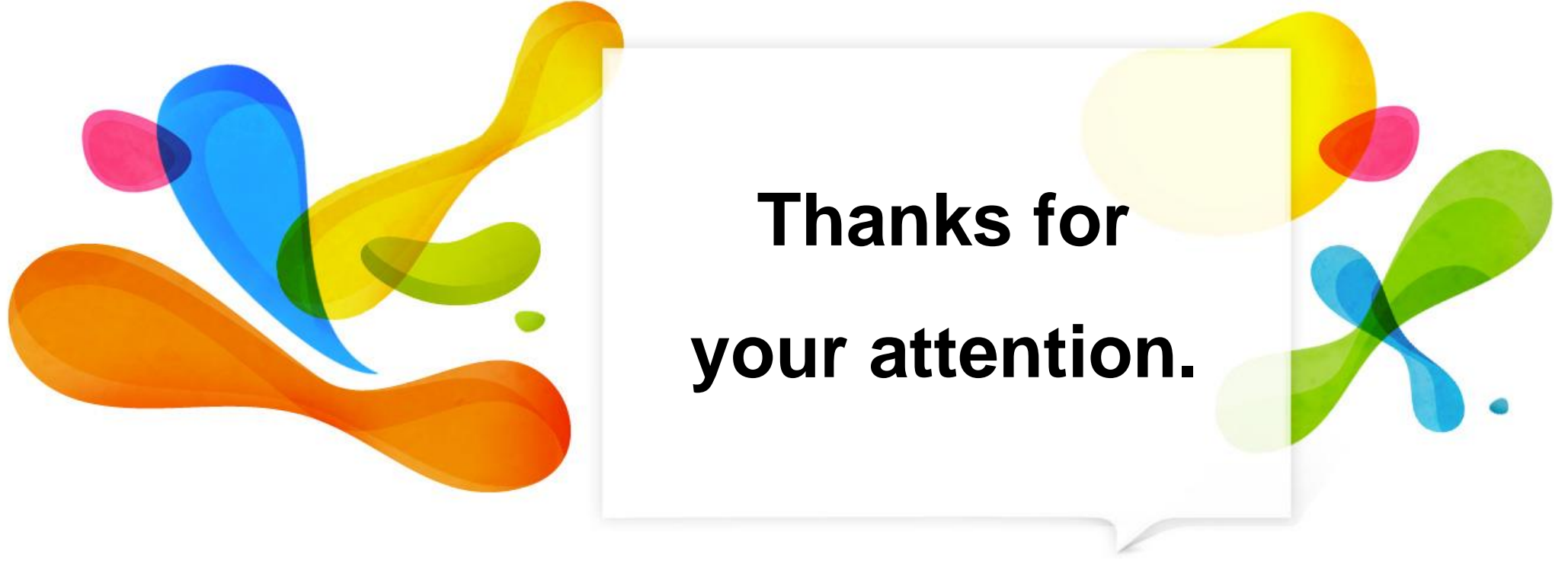
### - Comparison of cytotoxicity for HTPs and 3R4F

| Test Products | TPM  |                                 | GVP  |                                 |
|---------------|--|---------------------------------|--|---------------------------------|
|               | IC <sub>50</sub><br>( $\mu$ g of TPM<br>equivalent/mL) | Relative<br>Cytotoxicity<br>(%) | IC <sub>50</sub><br>( $\mu$ g of TPM<br>equivalent/mL) | Relative<br>Cytotoxicity<br>(%) |
| 3R4F          | 73 $\pm$ 2   | 100                             | 164 $\pm$ 26   | 100                             |
| Type A        | 520 $\pm$ 44   | 14 <b>86% ↓</b>                 | 1800 $\pm$ 97  | 9 <b>91% ↓</b>                  |
| Type B        | Can not be<br>calculated                               | Less than<br>7.3% <b>92% ↓</b>  | Can not be<br>calculated                               | Less than<br>3.3% <b>96% ↓</b>  |

- Cytotoxicity of test products was as follows; 3R4F >> HTP-A > HTP-B.



- ▶ Amount of harmful substances emitted by heated tobacco products was significantly lower than that of conventional cigarettes.
- ▶ In the Ames test, all heated tobacco products tested showed negative mutagenicity. Cytotoxicity of samples was as follows; 3R4F >> HTP-A > HTP-B.
- ▶ Smoke emission and cytotoxicity levels of HTP-B were much lower than those of HTP-A and it may be due to lower heating temperature of HTP-B.
- ▶ There are several limitations in applying cigarette-oriented experiments to heated tobacco products. Therefore, it seems to be necessary to develop an appropriate toxicity evaluation technology for NGPs (Next Generation products).



**Thanks for  
your attention.**