

Dose recommendations for 3-month smokeless tobacco feeding studies

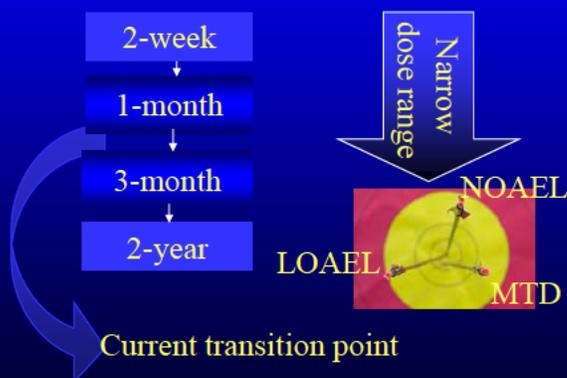
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Smokeless tobacco feeding studies

Goals

- Determine if Blend and Extract are different in rodent studies
- Define dose ranges progressively based on studies in rats and mice:



Definitions

- **NOAEL** = No Observed Adverse Effect Level = Dose that induces no observed effects
- **LOAEL** = Lowest Observed Adverse Effect Level = Dose that induces minimal adverse effects
- **MTD** = Maximum Tolerated Dose = Dose that animal can tolerate for majority of lifetime with no significant impairment/toxic effect other than carcinogenicity (typical MTD target: ~10% body weight↓)

Abbreviations used in this presentation

- C=control group (diet only, no tobacco added)
- N=nicotine tartrate group (positive control, diet with nicotine tartrate)
- B=tobacco blend group (diet with tobacco blend)
- E=water extract of tobacco blend group (diet with tobacco extract)
- M=males
- F=females
- All doses (e.g., 0.2, 2, 8, 200): expressed in mg nicotine/kg body weight/day
- TK=toxicokinetic study, run in parallel with core study
- Core=main study, separate from toxicokinetic study

2-Week studies

Rats

Mice

Test design

Mouse study design (original)

Group	Doses (mg Nicotine/kg body weight/day)	Number of male mice
1-Control (diet only, no tobacco)	0	5
2-Tobacco blend (low dose)	0.2	5
3-Tobacco blend (intermediate dose 1)	2	5
4-Tobacco blend (intermediate dose 2)	4	5
5-Tobacco blend (intermediate dose 3)	8	5
6-Tobacco blend (intermediate dose 4)	20	5
7-Tobacco blend (high dose)	40	5
8-Tobacco extract (low dose)	0.2	5
9-Tobacco extract (intermediate dose 1)	2	5
10-Tobacco extract (intermediate dose 2)	4	5
11-Tobacco extract (intermediate dose 3)	8	5
12-Tobacco extract (intermediate dose 4)	20	5
13-Tobacco extract (high dose)	40	5
14-Nicotine (low dose)	2	5
15-Nicotine (intermediate dose 1)	8	5
16-Nicotine (intermediate dose 2)	20	5
17-Nicotine (high dose)	40	5
18-Sentinel	0	10

Mouse study design (higher dose repeat)

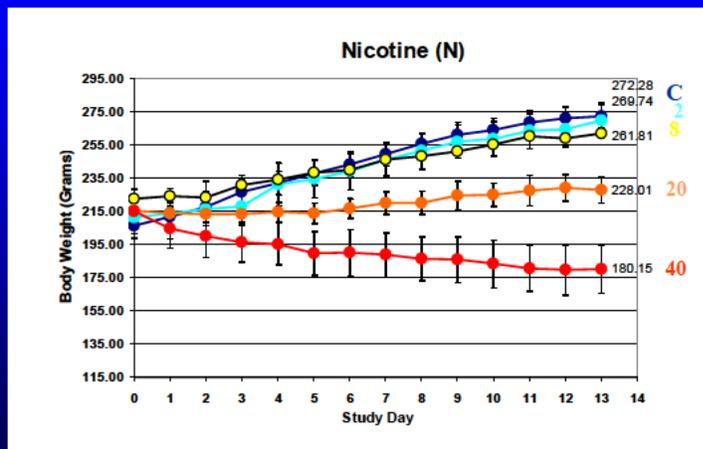
Group	Doses (mg Nicotine/kg body weight/day)	Number of male mice
1-Control (diet only, no tobacco)	0	10
2-Tobacco blend (low dose)	40	5
3-Tobacco blend (intermediate dose 1)	80	5
4-Tobacco blend (intermediate dose 2)	160	5
5-Tobacco blend (intermediate dose 3)	240	5
6-Tobacco blend (high dose)	400	5
7-Tobacco extract (low dose)	40	5
8-Tobacco extract (intermediate dose 1)	80	5
9-Tobacco extract (intermediate dose 2)	160	5
10-Tobacco extract (intermediate dose 3)	240	5
11-Tobacco extract (high dose)	400	5
12-Nicotine (low dose)	40	5
13-Nicotine (intermediate dose 1)	80	5
14-Nicotine (intermediate dose 2)	160	5
15-Nicotine (intermediate dose 3)	240	5
16-Nicotine (high dose)	400	5
17-Sentinel	0	10

Rat study design

Group	Doses (mg Nicotine/kg body weight/day)	Number of male rats
1-Control (diet only, no tobacco)	0	5
2-Tobacco blend (low dose)	0.2	5
3-Tobacco blend (intermediate dose 1)	2	5
4-Tobacco blend (intermediate dose 2)	4	5
5-Tobacco blend (intermediate dose 3)	8	5
6-Tobacco blend (intermediate dose 4)	20	5
7-Tobacco blend (high dose)	40	5
8-Tobacco extract (low dose)	0.2	5
9-Tobacco extract (intermediate dose 1)	2	5
10-Tobacco extract (intermediate dose 2)	4	5
11-Tobacco extract (intermediate dose 3)	8	5
12-Tobacco extract (intermediate dose 4)	20	5
13-Tobacco extract (high dose)	40	5
14-Nicotine (low dose)	2	5
15-Nicotine (intermediate dose 1)	8	5
16-Nicotine (intermediate dose 2)	20	5
17-Nicotine (high dose)	40	5
18-Sentinel	0	10

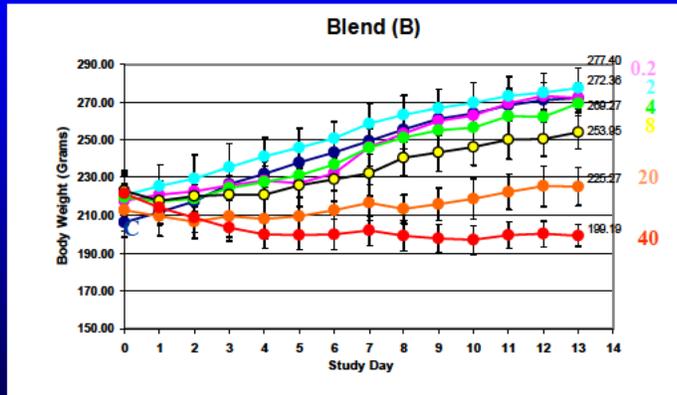
2-Week studies Results

Rats: body weights



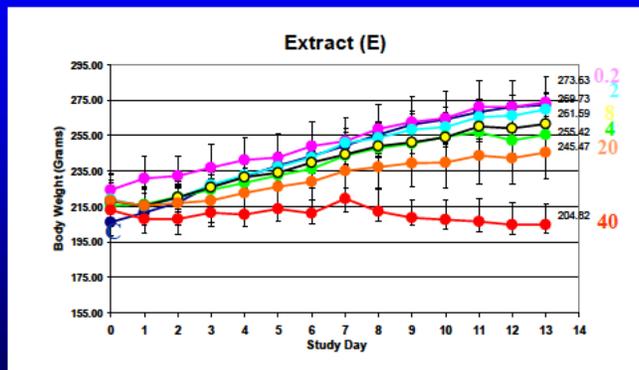
Dose
range of
interest
1-month
study:
0.2-20

Rats: body weights



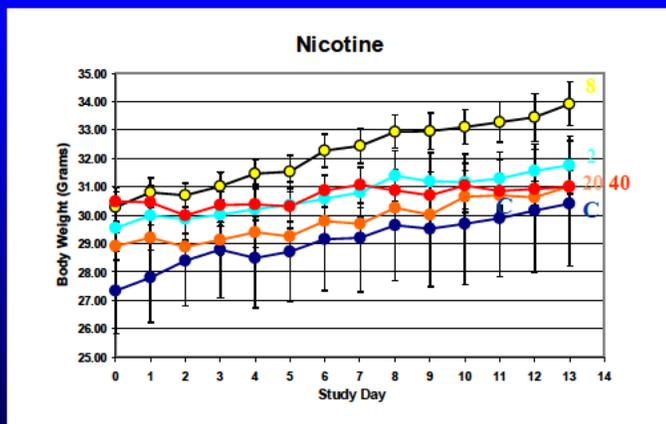
Dose range of interest
1-month study:
0.2-20

Rats: body weights



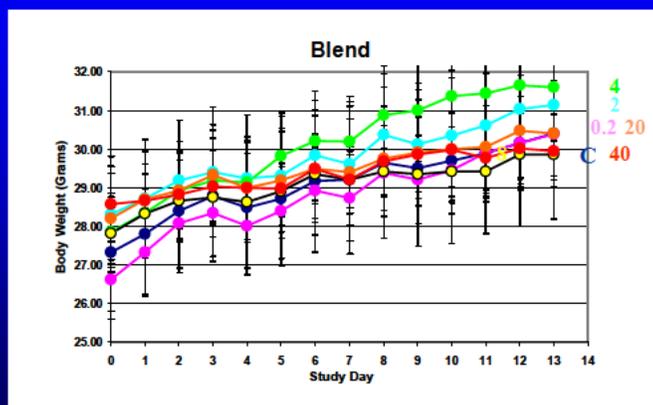
Dose range of interest
1-month study:
0.2-20

Mice (original): body weights



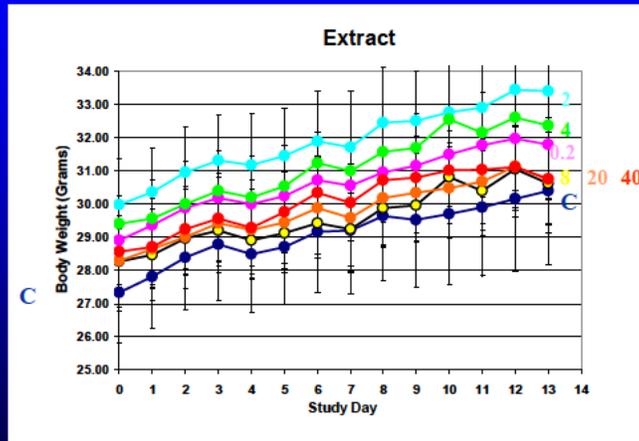
- No clear dose-response
- Sensitivity rats>mice

Mice (original): body weights



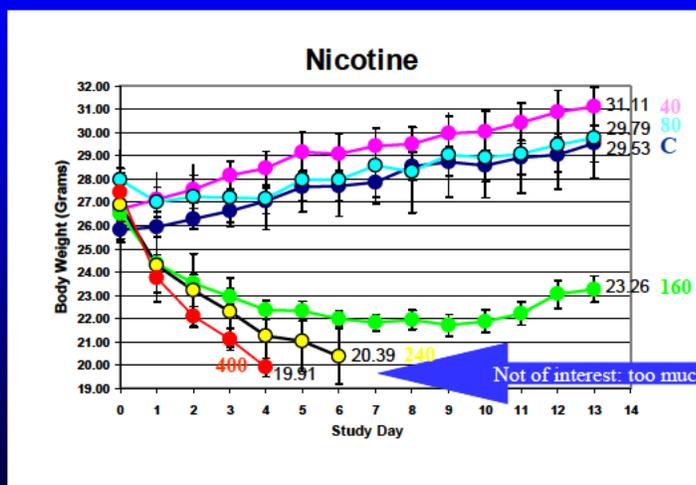
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Mice (original): body weights



- No clear dose-response
- Sensitivity rats>mice

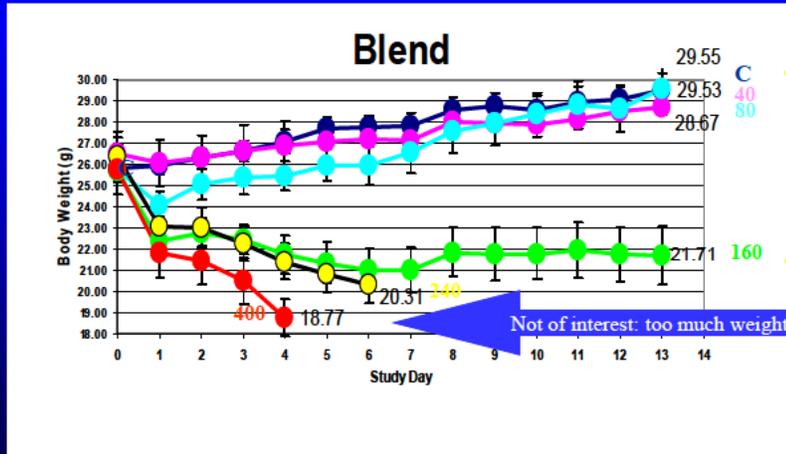
Mice (repeat): body weights



Dose range of interest 1-month study: 40-160

Not of interest: too much weight loss

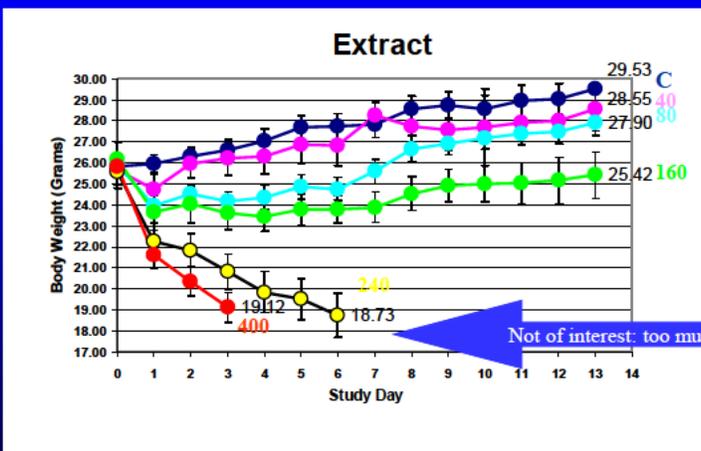
Mice (repeat): body weights



Dose range of interest
1-month study:
40-160

Not of interest: too much weight loss

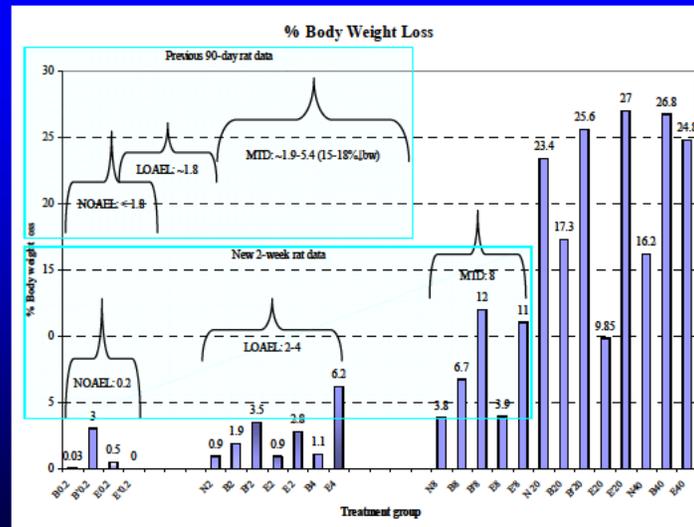
Mice (repeat): body weights



Dose range of interest
1-month study:
40-160

Not of interest: too much weight loss

Rats 2-week=>1-month



Final doses for 1-month studies

Test article	Rats	Mice
Nicotine	20	200
Blend, extract	0.2	2
	2	20
	8	80
	20	200

Note: Rats more sensitive than mice

1-Month studies

Rats

Mice

Test design

Rat study design

Group	Target Dose (mg Nicotine/ kg/day)	Number of Rats			
		Males		Females	
		Core	TK ^a	Core	TK ^a
1-Control	0	10	--	10	--
2-Nicotine Tartrate (High Dose)	20	10	6	10	6
3-Control Diet Pair-fed to Nicotine Tartrate (High Dose)	0	10	--	10	--
4-Tobacco Blend (Low Dose)	0.2	10	6	10	6
5-Tobacco Blend (Intermediate Dose 1)	2	10	6	10	6
6-Tobacco Blend (Intermediate Dose 2)	8	10	6	10	6
7-Tobacco Blend (High Dose)	20	10	6	10	6
8-Control Diet Pair-fed to Tobacco (High Dose)	0	10	--	10	--
9-Tobacco Extract (Low Dose)	0.2	10	6	10	6
10-Tobacco Extract (Intermediate Dose 1)	2	10	6	10	6
11-Tobacco Extract (Intermediate Dose 2)	8	10	6	10	6
12-Tobacco Extract (High Dose)	20	10	6	10	6
13-Control Diet Pair-fed to Tobacco Extract (High Dose)	0	10	--	10	--
14-Sentinels	0	10	--	10	--

^a Nicotine/cotinine analysis

Mouse study design

Group	Target Dose (mg Nicotine/ kg/day) ^a	Number of Mice			
		Males		Females	
		Core	TK ^a	Core	TK ^a
1-Control	0	10	--	10	--
2-Nicotine Tartrate High Dose	200	10	23	10	23
3-Tobacco Blend (Low Dose)	2	10	23	10	23
4-Tobacco Blend (Intermediate Dose 1)	20	10	23	10	23
5-Tobacco Blend (Intermediate Dose 2)	80	10	23	10	23
6-Tobacco Blend (High Dose)	200	10	23	10	23
7-Tobacco Extract (Low Dose)	2	10	23	10	23
8 - Tobacco Extract (Intermediate Dose 1)	20	10	23	10	23
9-Tobacco Extract (Intermediate Dose 2)	80	10	23	10	23
10-Tobacco Extract (High Dose)	200	10	23	10	23
11-Sentinels	0	10	--	10	--

^a Nicotine/cotinine analysis

Endpoints measured

- Body weights
- Food consumption
- Clinical observations
- Clinical pathology (clinical chemistry, hematology)
- Neurotoxicity
- Organ weights
- Toxicokinetics (plasma nicotine, cotinine)

Neurotoxicity endpoints: rats and mice

•Home cage

- Posture
- Tremor
- Convulsions
- Lethargy, arousal
- Eyelid closure

•While handling

- Ease of removing
- Ease of handling
- General condition
- Stains (urine, feces)
- Salivation
- Piloerection
- Fur appearance
- Vocalization
- Lacrimation
- Palpebral reflex
- Pupil response

•Open field

- Lines crossed
- Rearings
- Urine pools, fecal boluses
- Posture
- Tremor
- Convulsion
- Gait
- Stereotypy
- Bizzare behavior
- Vocalizations

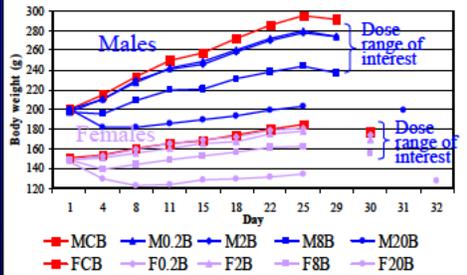
•Reflexes

- Approach response
- Touch
- Startle
- Tail pinch
- Paws on grid
- Grip strength
- Righting reflex
- Rectal temperature

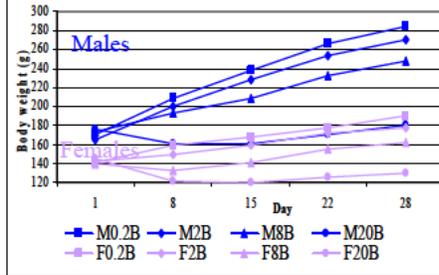
1-Month studies Results

Rats: body weights Blend

Core

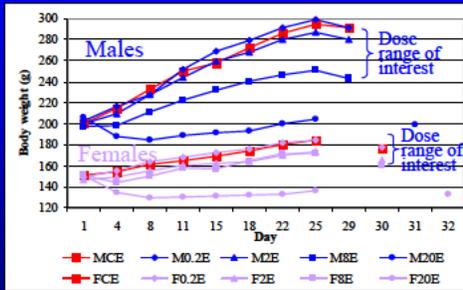


TK

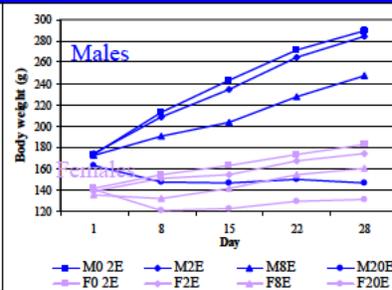


Rats: body weights Extract

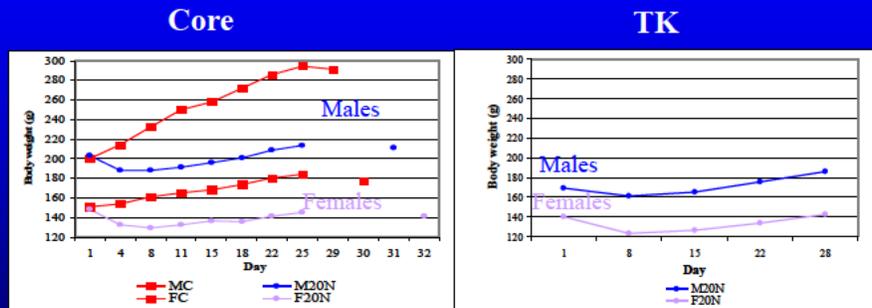
Core



TK



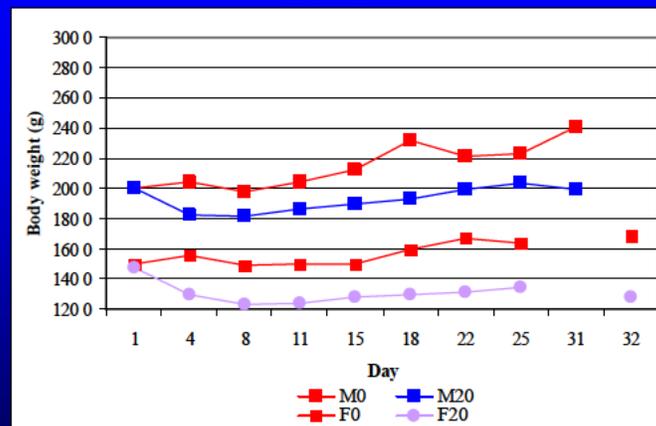
Rats: body weights Nicotine



20 mg/kg/day not of interest:

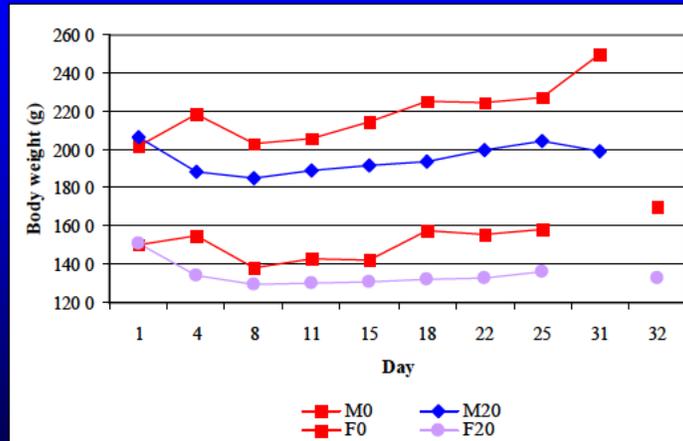
- too much body weight loss

Rats: body weights (paired feeding) Blend



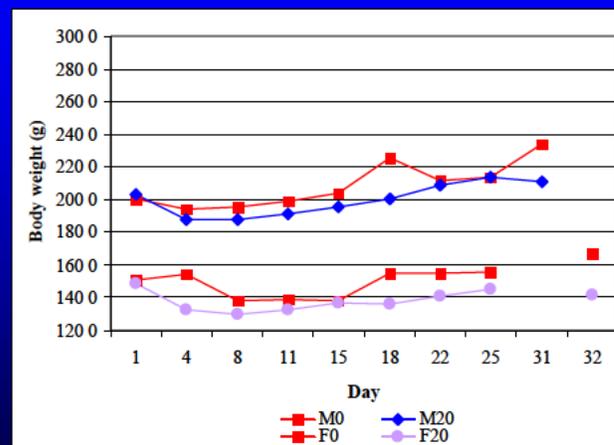
- Paired feeding = treatment groups fed same amount of diet as Control
- Food restricted treatment group: ↓ body weights
- Test article induces its own effect (including nicotinic effects-appetite suppression)

Rats: body weights (paired feeding) Extract



- Paired feeding = treatment groups fed same amount of diet as Control
- Food restricted treatment group: ↓body weights
- Test article induces its own effect (including nicotinic effects-appetite suppression)

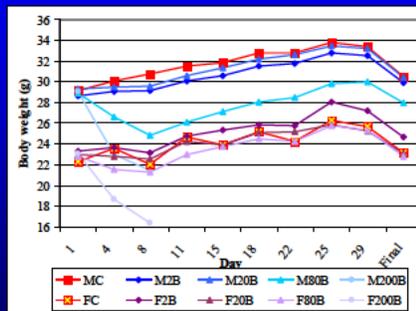
Rats: body weights (paired feeding) Nicotine



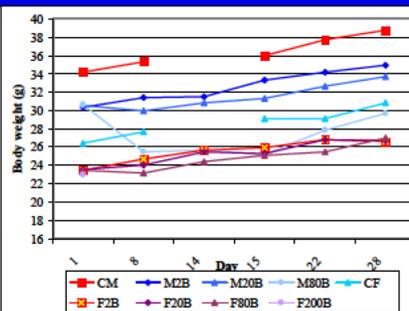
- Paired feeding = treatment groups fed same amount of diet as Control
- Food restricted treatment group: ↓body weights
- Positive control (nicotine) induces its own effect (e.g., appetite suppression)

Mice: body weights Blend

Core



TK

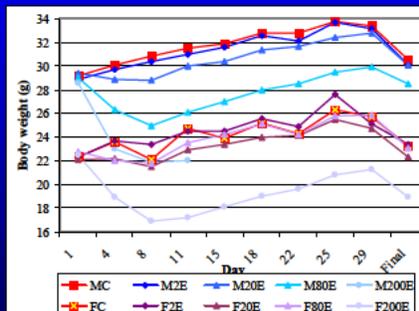


Dose ranges of interest

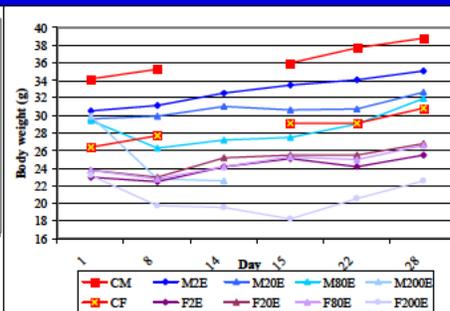
- Males: 2-80 mg/kg/day
- Females: 2-120 mg/kg/day

Mice: body weights Extract

Core



TK

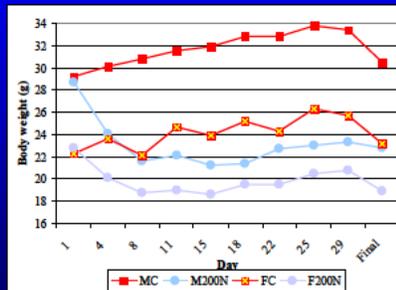


Dose ranges of interest

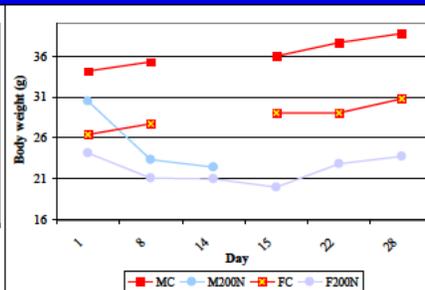
- Males: 2-80 mg/kg/day
- Females: 2-120 mg/kg/day

Mice: body weights Nicotine

Core



TK



Dose range of interest

- Does NOT include 200 mg/kg/day
- Too much body weight loss

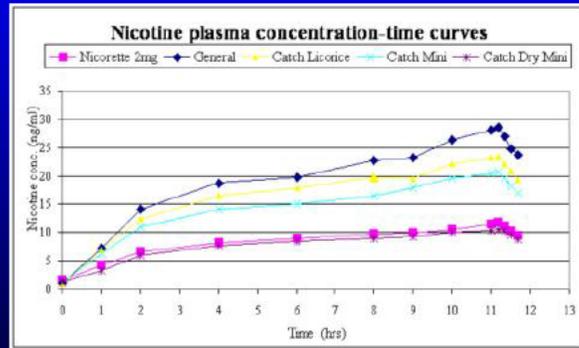
Nicotine and cotinine in humans

Parameter	Smoking	Smokeless
Nicotine absorption	Faster	Slower
Plasma nicotine	Abrupt decrease after exposure	Plateau during and after exposure consistent with continuous absorption even when tobacco is removed
Nicotine	T _{1/2} =2-3 hr	Accumulation over 6-8 hr with continued use
Cotinine	T _{1/2} =15-17 hr	
Typical plasma nicotine steady state (ng/ml)	~10-50	~10-50
Typical plasma cotinine (ng/ml)	10->900 (average 250-300)	14-900 (average ~200-300)

Benowitz NL (1993) Smokeless tobacco control Monograph No 2
 Davis RA and Curvall M (1999) Analytical determination of nicotine, Gorrod JW, Jacob P III, eds, Elsevier, Amsterdam
 Centers for Disease Control and Prevention (2005) Third national report on human exposure to environmental chemicals

Nicotine plasma levels: smokeless exposure humans Example

- Lunell et al., 2005
- Blood plasma concentration-time (4 snus brands and a 2 mg nicotine chewing gum):

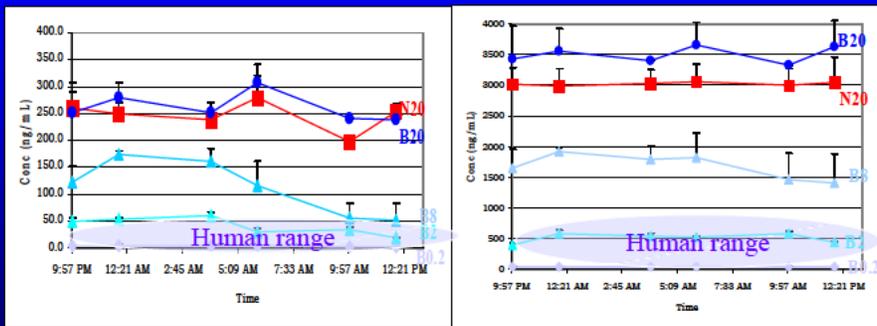


<http://www.gothiatek.com>

Rats: toxicokinetics, 2-weeks, males Blend

Nicotine

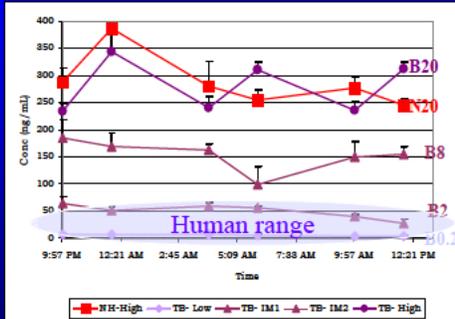
Cotinine



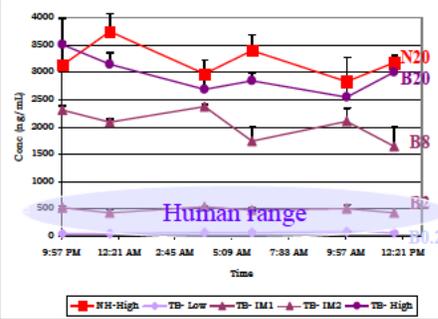
- Cmax & tmax variability allows selection of > 1 time point
- Dose-plasma level concordance: \uparrow dose \Rightarrow \uparrow plasma level

Rats: toxicokinetics, 2-weeks, females Blend

Nicotine



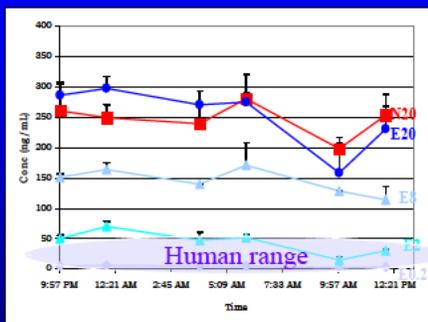
Cotinine



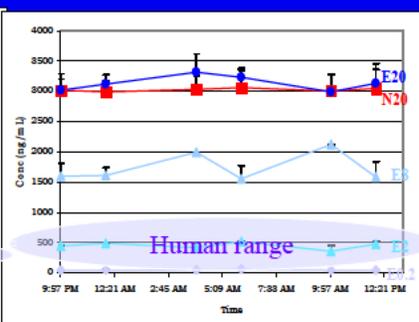
- Cmax & tmax variability allows selection of > 1 time point
- Dose-plasma level concordance: ↑ dose => ↑ plasma level

Rats: toxicokinetics, 2-weeks, males Extract

Nicotine



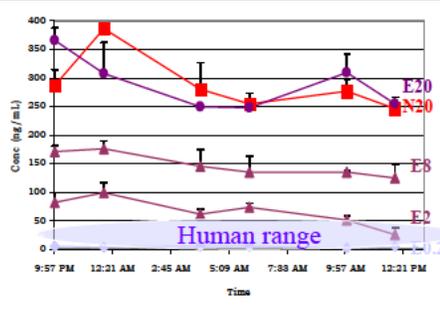
Cotinine



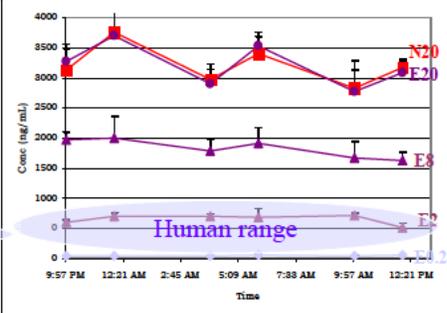
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Rats: toxicokinetics, 2-weeks, females Extract

Nicotine



Cotinine



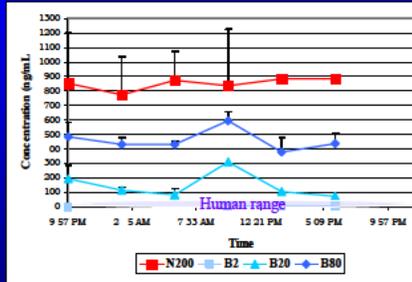
- C_{max} & t_{max} variability allows selection of > 1 time point
- Dose-plasma level concordance: ↑ dose ⇒ ↑ plasma level

Rats toxicokinetics: Sampling time (subsequent studies)

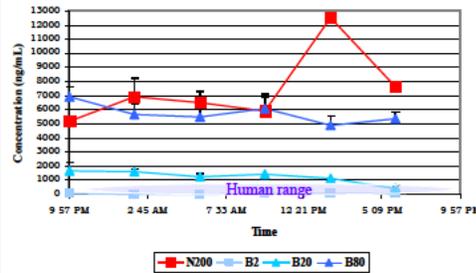
- 1 sampling time: 12 AM
 - Males and females
 - Nicotine and cotinine
- Rationale:
 - Variability in c_{max} and t_{max} allows selection flexibility
 - Recommended time point is:
 - most consistently re-occurring peak time point or
 - closest to the peak time point
 - Practical considerations (1 time point vs. 2)

Mice: toxicokinetics, 2-weeks, males Blend

Nicotine



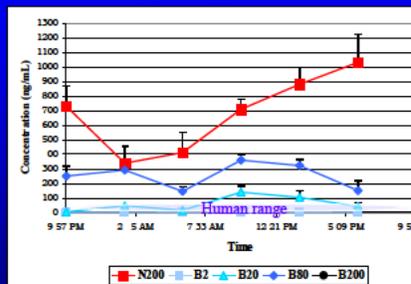
Cotinine



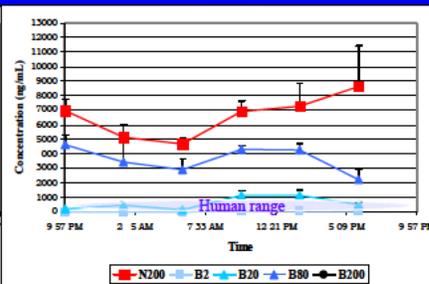
- Cmax & tmax variability allows selection of > 1 time point
- Dose-plasma level concordance: \uparrow dose \Rightarrow \uparrow plasma level

Mice: toxicokinetics, 2-weeks, females Blend

Nicotine



Cotinine

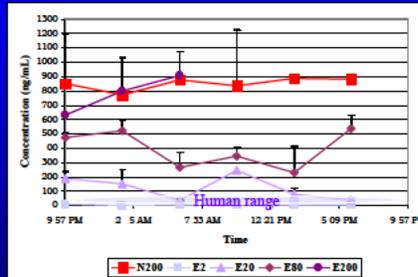


- Cmax & tmax variability allows selection of > 1 time point
- Dose-plasma level concordance: \uparrow dose \Rightarrow \uparrow plasma level

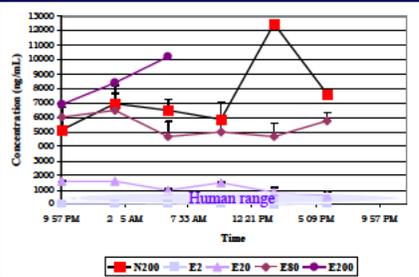
Mice: toxicokinetics, 2-weeks, males

Extract

Nicotine



Cotinine

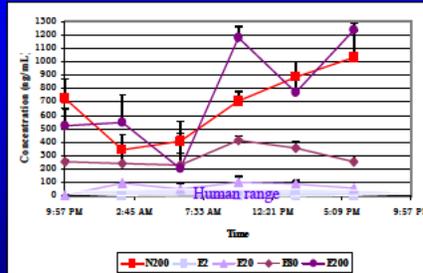


- Cmax & tmax variability allows selection of > 1 time point
- Dose-plasma level concordance: \uparrow dose \Rightarrow \uparrow plasma level

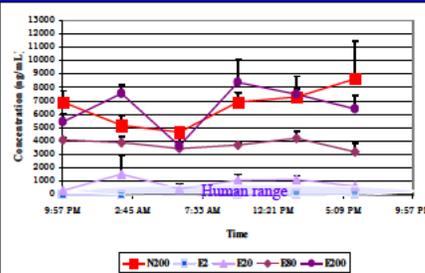
Mice: toxicokinetics, 2-weeks, females

Extract

Nicotine



Cotinine



- Cmax & tmax variability allows selection of > 1 time point
- Dose-plasma level concordance: \uparrow dose \Rightarrow \uparrow plasma level

Mice toxicokinetics: Sampling time (subsequent studies)

- 1 sampling time: 10 AM
 - Males and females
 - Nicotine and cotinine
- Rationale:
 - Variability in c_{max} and t_{max} allows selection flexibility
 - Recommended time point is:
 - most consistently re-occurring peak time point or
 - closest to the peak time point
 - Practical considerations (1 time point vs. 2)

Clinical signs

- **Rats**
 - Generally, no significant treatment-related clinical signs
- **Mice**
 - Clinical signs occurred mostly in the high dose (200 mg/kg/day) groups
 - Blend
 - Extract
 - Nicotine
 - Males more sensitive than females

Neurotoxicity

■ Rats

- Only significant treatment-related effect: ↓ temperature
 - Only females (high dose/20)
 - Blend
 - Extract
 - Nicotine

■ Mice

- Only significant treatment-related effect: ↓ temperature
 - Males^a
 - Nicotine
 - Females^b
 - Nicotine^c
 - Extract^d

^aNo high dose (200) male blend or extract groups left

^bNo high dose (200) female blend group left

^cN=10 female high dose (200) nicotine group; N=6 male high dose (200) nicotine

^dN=4 female high dose (200) extract group

Consistency of findings with literature data

- Body temperature is modulated by brain nicotinic acetylcholine receptors
- Nicotine is known to cause hypothermia in rats, mice, and humans
- Chronic nicotine treatment generally results in tolerance to several actions of nicotine, including hypothermia-related effects

Ruskin DN, Anand R, LaHoste GJ (2007) Menthol and nicotine oppositely modulate body temperature in the rat
Eur J Pharmacol 559(2-3):161-4

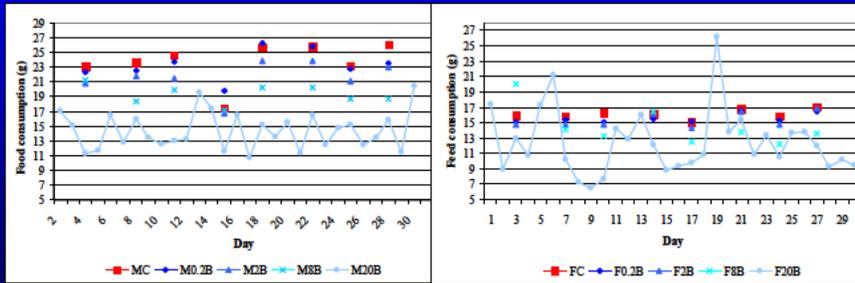
Levin ED (2001) *Nicotinic Receptors in the nervous system* CRC Press

Benowitz NL, Porchet H, Jacob III P (1990) *Nicotine psychopharmacology: Molecular, cellular, and behavioural aspects* Wonnacott S, Russell MAH, Stolerman IP, eds., Oxford University Press, Oxford

Food Consumption: Rats Blend

Males

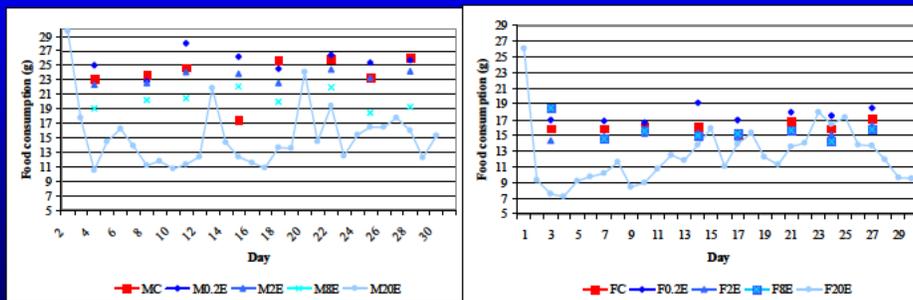
Females



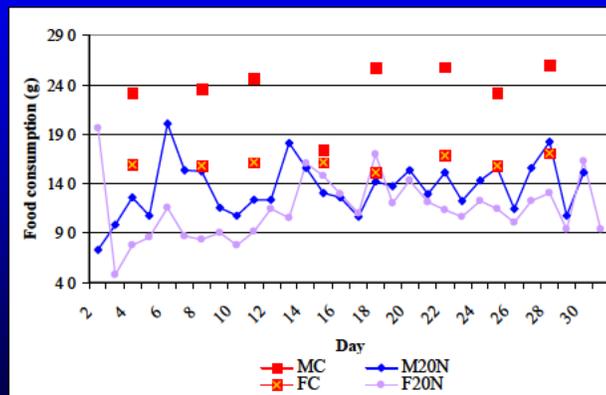
Food Consumption: Rats Extract

Males

Females



Food Consumption: Rats Nicotine Males and Females



Organ weights (necropsy)

■ Rats

- Some statistically significant organ weight changes (absolute, % organ/body weight) were noted in the two highest dose groups
- Pair-fed groups: no organ weight-related differences

■ Mice

- Some statistically significant organ weight changes (absolute, % organ/body weight) were noted in the two highest dose groups
- More changes in Nicotine group than Blend or Extract
- No clear dose-response and no clear target organ apparent

Clinical pathology

- **Rats**

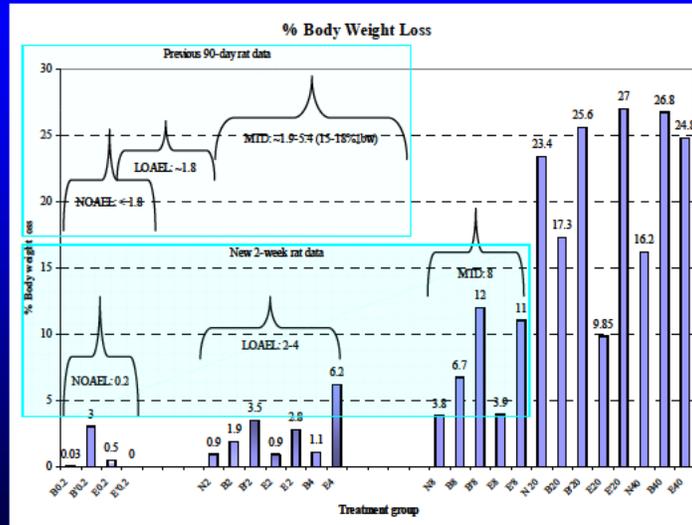
- No significant treatment-related effects

- **Mice**

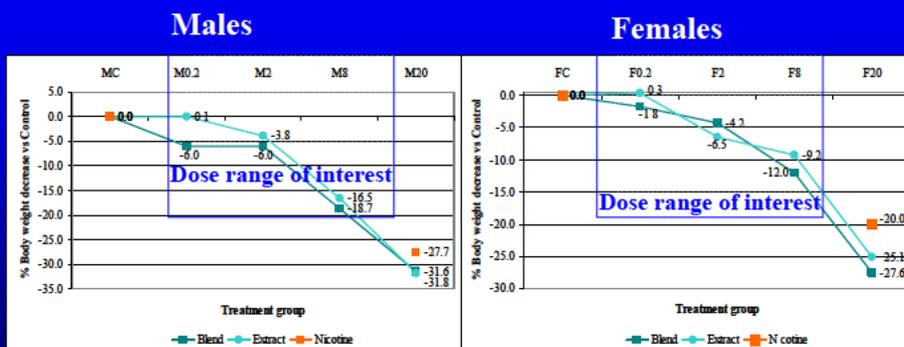
- Some effects in highest dose groups (80 and 200)
 - Nicotine 200 mg/kg/day, males 80 mg/kg/day extract, females 200 mg/kg/day extract:
 - ↓ total white cell and total lymphocyte counts (likely treatment-related)
 - ↓ hemoglobin (not clear if treatment-related)
 - Nicotine 200 mg/kg/day (few males, females)
 - ↑ aspartate aminotransferase (not clear if treatment-related)

Summary

Rats 2-weeks=>1-month=>3-months

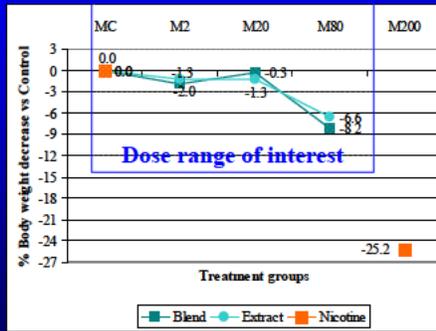


Rats: 1-month studies (end) % Body weight decrease

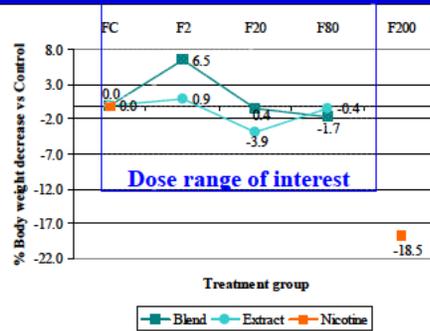


Mice: 1-month studies (end) % Body weight decrease

Males



Females



Possible dose ranges for 90-day studies

Rats 0.2 2 4 6 8 10 12

Mice 2 4 6 8 20 40 60 80 100 120

Doses considered

<i>Test article</i>	<i>Dose class</i>	<i>Dose (mg Nicotine/kg/day)</i>	
		<i>Rats</i>	<i>Mice</i>
Nicotine	MTD	6-8	80-120 males 100-120 females
Blend, extract	NOAEL	0.2-0.3	2-8
	LOAEL	2-3	20-80
	MTD	6-8	80-120 males 100-120 females

Recommended doses for 90-day studies (mg nicotine/kg body weight/day)

Rats	NOAEL	LOAEL	MTD
Males			
Females	0.3	3	6

Mice	NOAEL	LOAEL	MTD
Males			
Females	6	60	120

Dose selection considerations

- Aim for a classical toxicology study design
- **Low dose**
 - Targeted NOAEL inclusion to:
 - Facilitate risk assessment
 - Have at least one dose relevant to humans (per plasma nicotine)
- **Intermediate dose**
 - Targeted LOAEL inclusion to:
 - Facilitate risk assessment
 - Have 2nd dose just above or within range relevant to humans (per plasma nicotine)
- **High dose**
 - Targeted MTD to ensure that:
 - Most animals are likely to stay on study (high but minimize losses)
 - High enough to obtain ~10% body weight reduction

Next steps: Start 90-day studies

- Finalize protocols (include doses): 8/15/08
- Mice animal receipt: 8/19/08
- Rat animal receipt: 8/26/08

On/ahead of schedule