May neonicotinoid insecticides cause neurodevelopmental disorder by environmental exposure?

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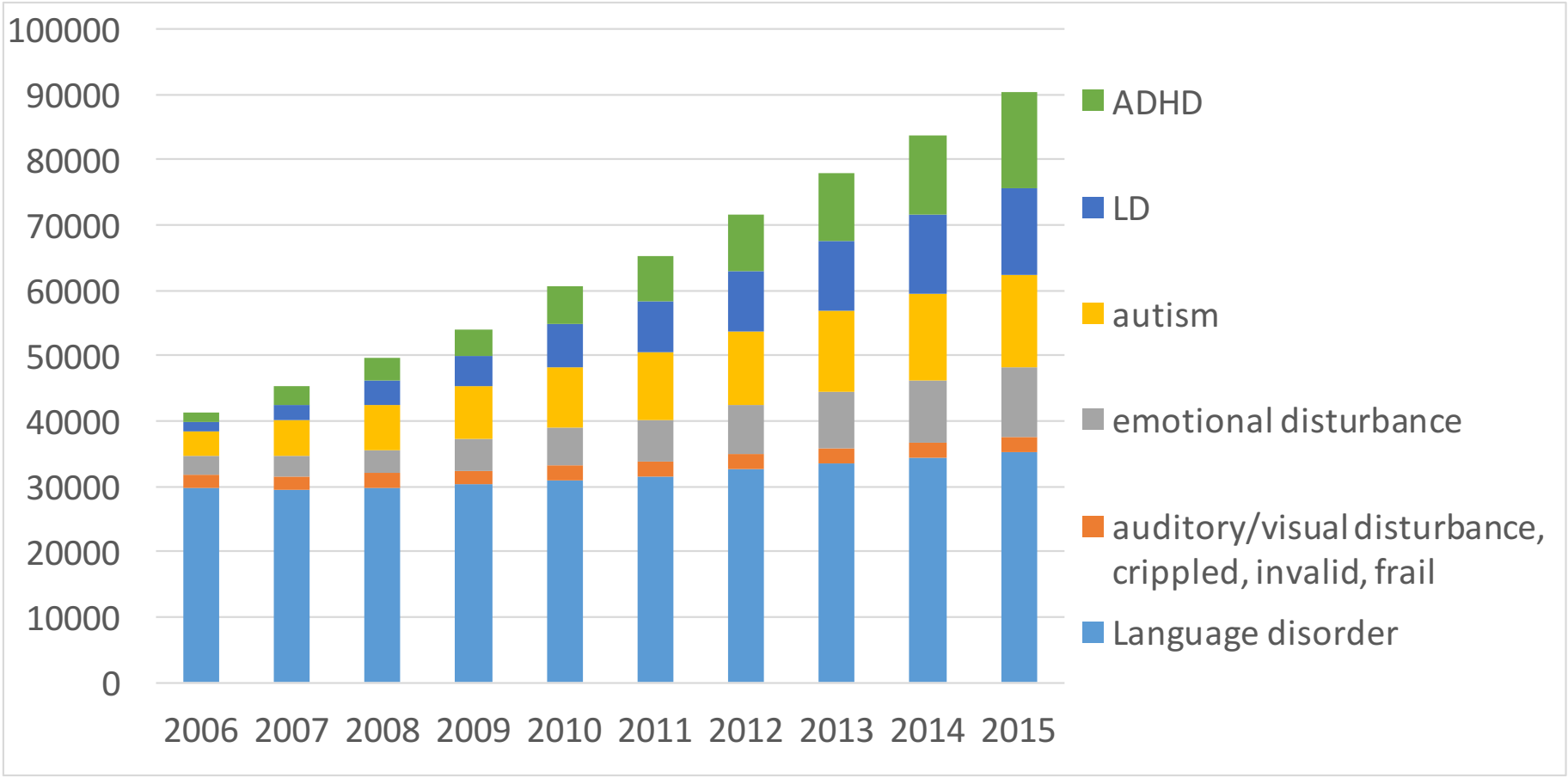
Today's message

- Neurodevelopmental Disorders may be caused by not only genetic factors, but also environmental chemicals, such as air pollutant, metal, and pesticides.
- Neonicotinoid insecticides (neonics) are world best selling systemic pesticides, nicotinic acetylcholine receptor agonist.
- **Neonics might cause neurodevelopmental disorders by environmental exposure**, because in vitro, animal, and clinical studies suggest it, recently.
- We have few evidence that neurodevelopmental disorders are not increasing by use of neonicotinoids.
- To keep natural child neurodevelopment, to revise acceptable dose of intake of neonicotinoids are one of the rational ways.

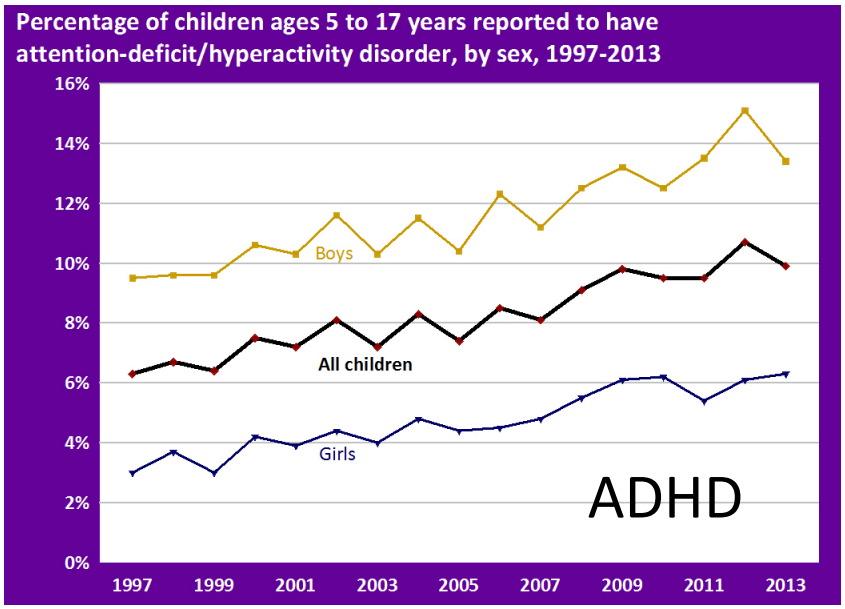
- I have no COI with regard to our presentation.

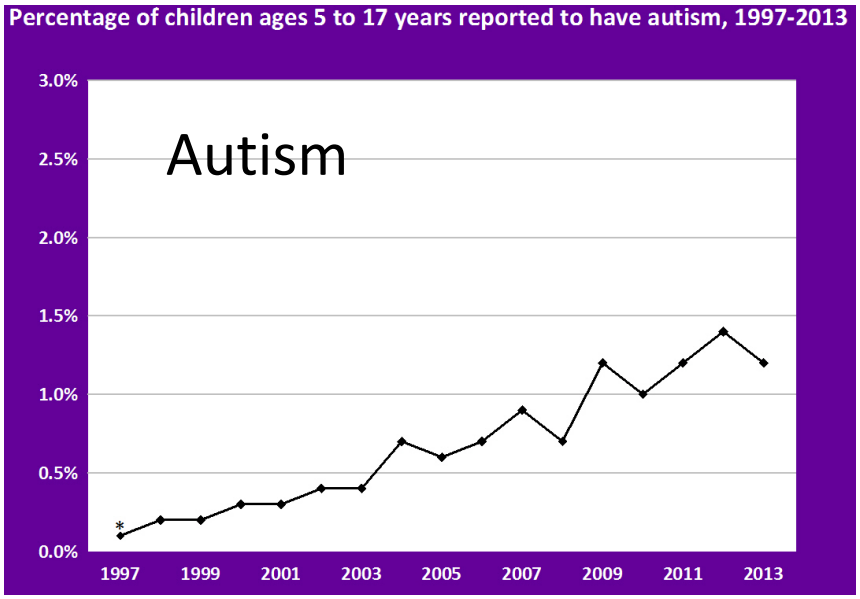
Children (6-14 years old) with special need by neurodevelopmental disorders in Japan

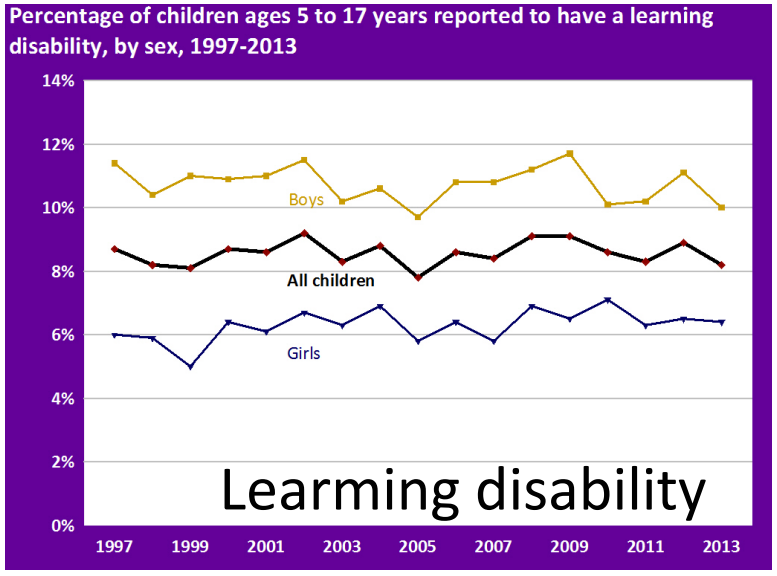
- **Consistently increasing in these ten years (Ministry of Education, Culture, Sports, Science and Technology, 2015).**

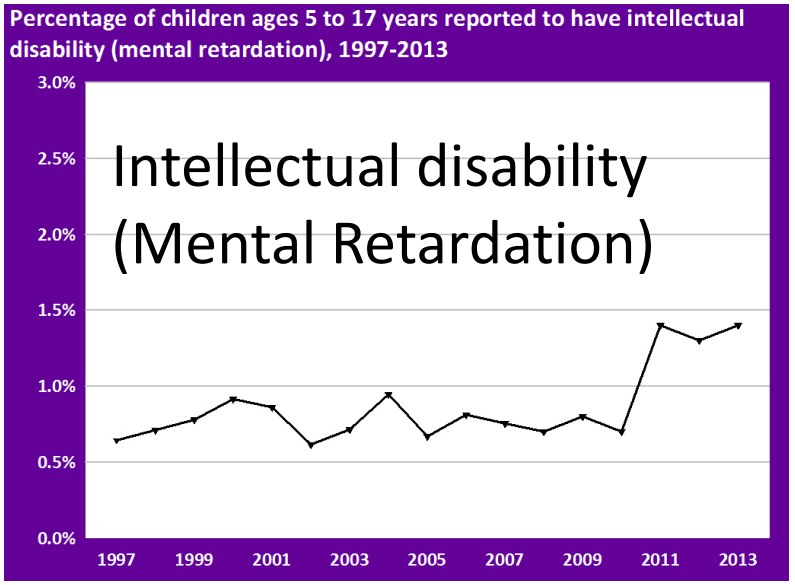


EPA also observe the same phenomena in 2015









The cause of neurodevelopmental disorders (EPA 2015)

- Attention-Deficit/Hyperactivity Disorder (ADHD)
  - Genetic factor
  - Maternal smoking during pregnancy
  - Preterm birth, Low birth weight, Psychosocial adversity
  - Lead, PCB, Phthalate, Organophospahte pestidies, Perfluorinated chemicals, Mercury
- Learning disablity
  - Genetic factor
  - Problem during pregnancy
  - Lead, Tabacco Smoke, Mercury, PCB
- Autism Spectrum Disorders
  - Genetic factor
  - Pesticide, Mercury, Air Pollutant, Phthalates
- Intellectual Disability (Mental Retardation)
  - genetic disorders, traumatic injuries, and prenatal events
  - Lead, mercury, PCB, organophosphate pesticides, PBDEs, phthalates, PAHs

Neurodevelopmental Disorders may be caused by pesticides

- **Insecticides are neurotoxic.**
- **Several epidemiological study showed positive relationships between environmental pesticide exposure and neurodevelopmental disorders.**
- **However, the data about neonicotinoids are rare.**

	Neuronal effect site	ADHD	autism	Developmental delay
Organochlorines	Na+ channel	+	+	+
Organophosphates	Enzyme	+	+	+
Pyrethroids	Na+ channel	+	+	+
Neonicotinoids	Receptor		?	

Neonicotinoids are systemic insecticides.

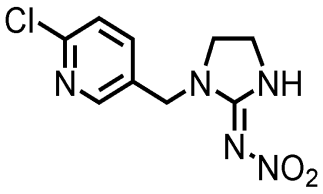
- Long lasting in plant, famous as honey bee's risk.
- Nicotinic acetylcholine receptor (nAChR) agonists

Halogenated

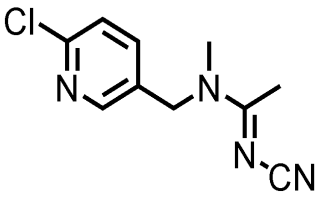
Chlorinated (organochlorine)

Chloropyridinyl

Imidacloplid

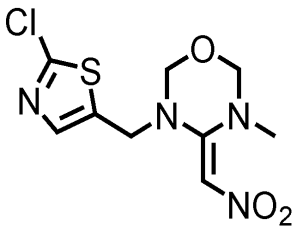


Acetamiprid



Chlorothiazolyl

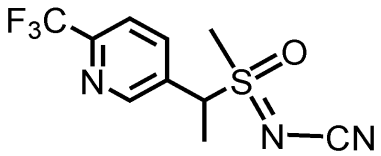
Thiamethoxam



Fluorinated

Sulfoximine

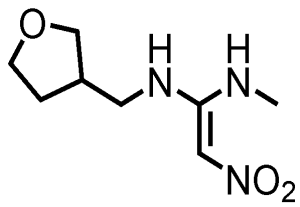
Sulfoxaflor



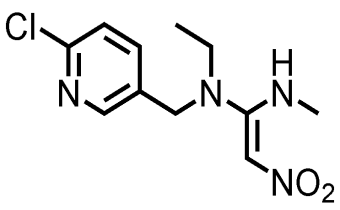
Non-halogenated

Tetrahydrofuranyl

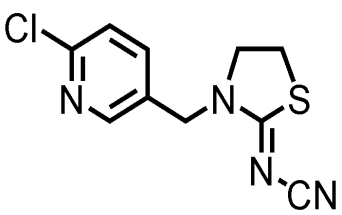
Dinotefuran



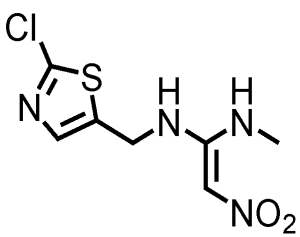
Nitenpyram



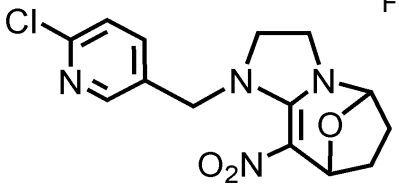
Thiacloprid



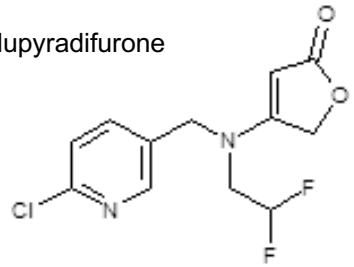
Clothianidin



Cycloxaprid

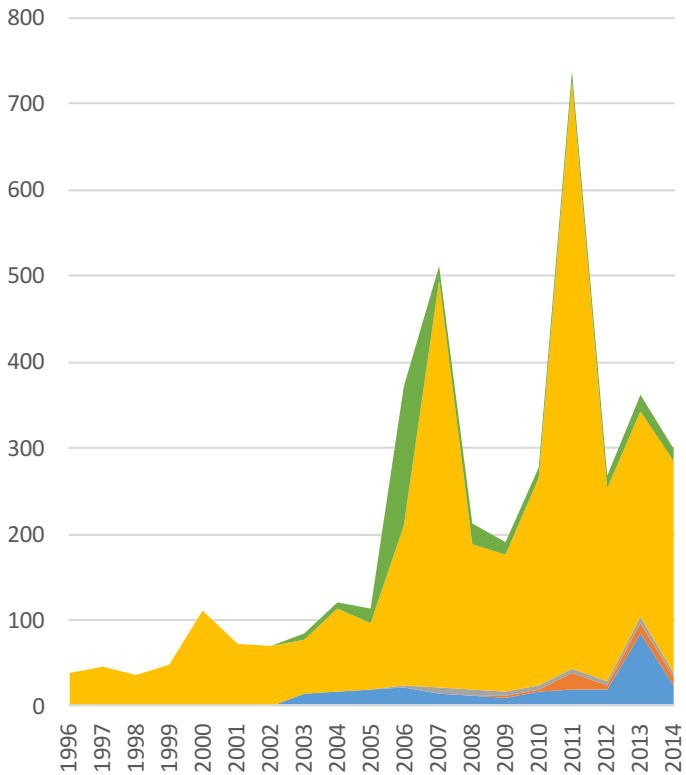


Flupyradifurone



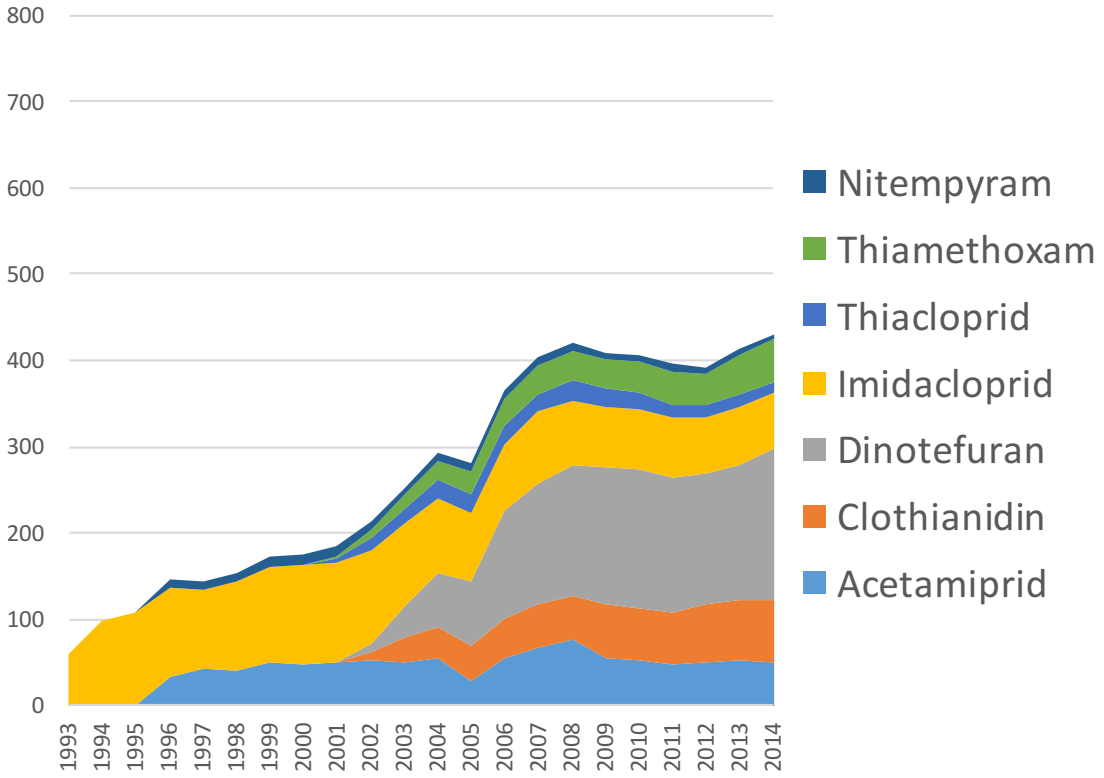
Shipment of neonicotinoids (tons)

CA=424,000 km^2



STATE OF CALIFORNIA
PESTICIDES SOLD IN CALIFORNIA
FOR YEAR: 1996-2014

JAPAN=378,000 km^2



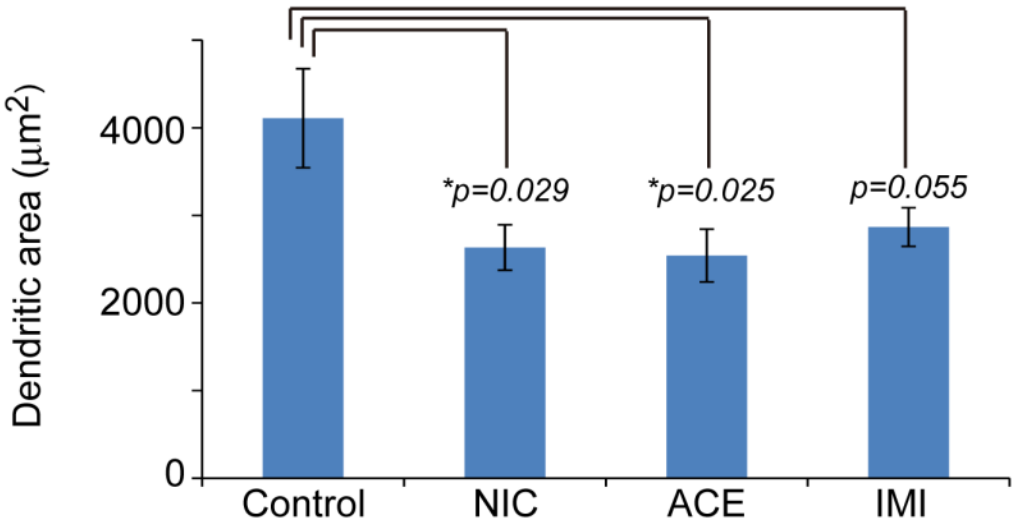
Database of National Institute for Environmetal Studies: 1993-2014

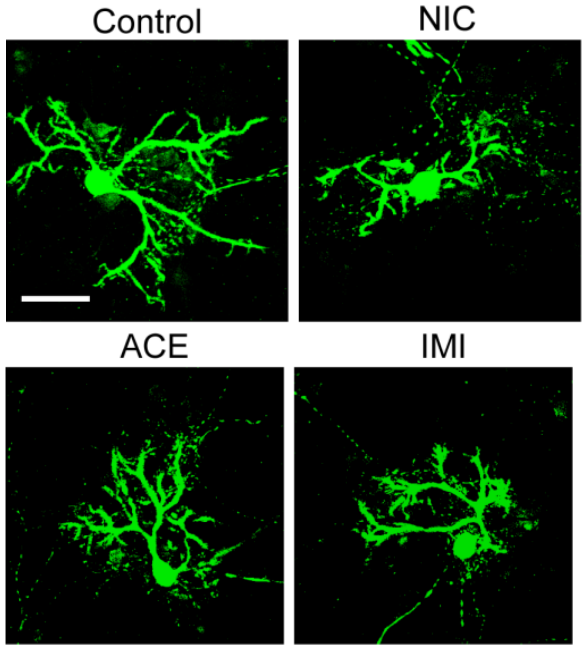
nAChRs have critical role in neurodevelopment.

- In the human fetal cerebellum, α4, α7, β2, and β4 nAChRs are highly expressed (Hellström-Lindahl, 1998).
- Endogenous cholinergic signaling via nAChRs is important in determining the morphological and functional maturation of neural circuit formation (Miwa, 2011).
- Glutamatergic synapse formation is promoted by α7-containing nAChRs and affected by nicotine exposure in hippocampal and cortical neurons (Lozada, 2012).
- Retinal β2 nAChRs are necessary for visual circuit formation (Burgridge, 2014)
- Prenatal nicotine exposure alters the visual cortex system in baboons (Duncan, 2015).
- Even at a dose lower than that necessary to activate the receptor, nicotine causes desensitization of nAChRs (Wang, 2005), which results in a disturbance of normal synapse formations at the developmental stage (Slotkin, 2016).

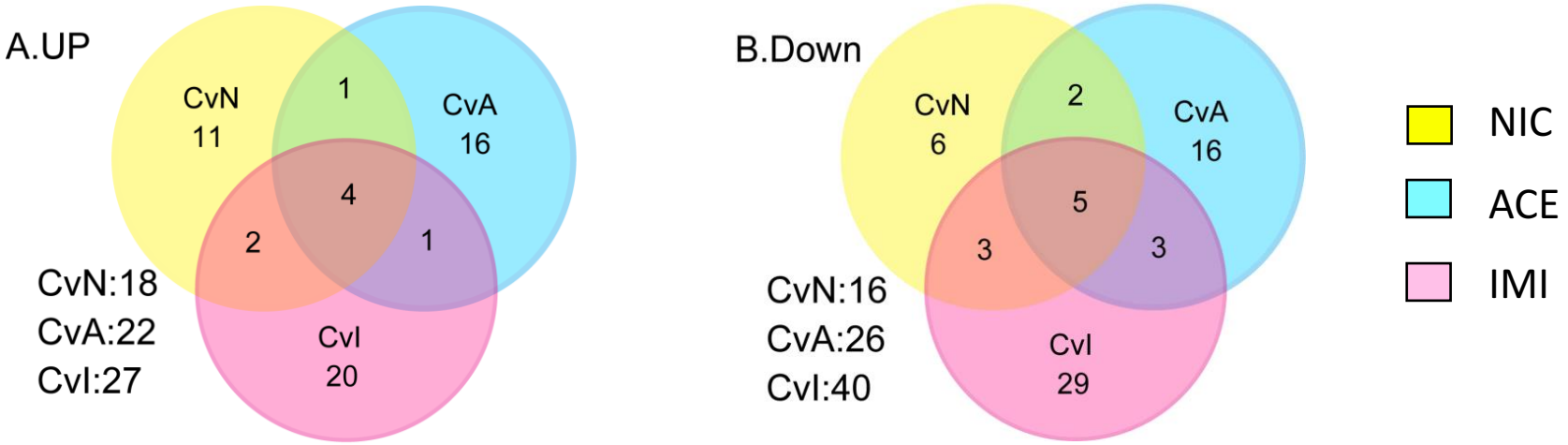
Acetamiprid and Imidacloprid Alter the Gene Expression Profile of Neuron-Enriched Cultures from Neonatal Rat Cerebellum (Kimura-Kuroda 2016)

- long- term (14 days) exposure of neuron-enriched cultures
- low dose (1 µM) nicotine, acetamiprid or imidacloprid.
- A slight disturbance in Purkinje cell dendritic arborization was observed in the exposed cultures.





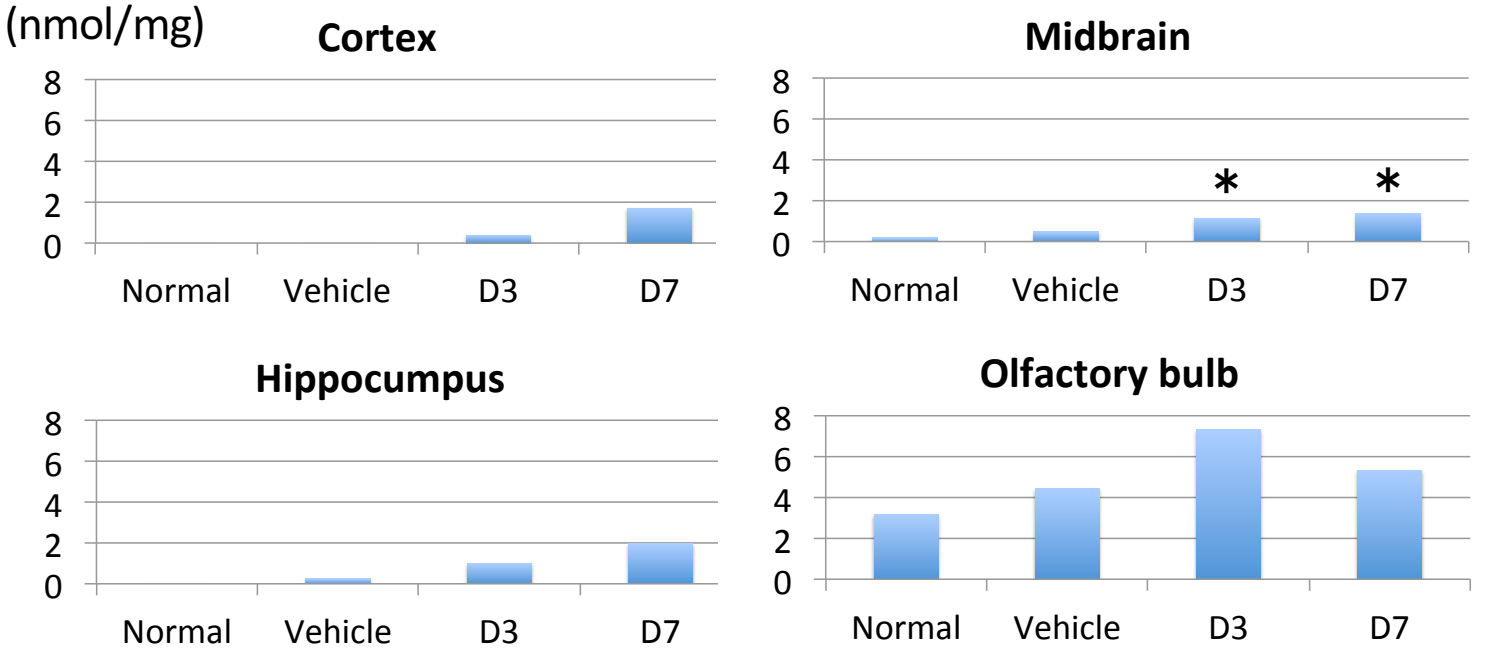
- Moreover,
- Significant differential expression ($p<0.05$, $q<0.05$, ≥1.5 fold) between control cultures versus nicotine-, acetamiprid-, or imidacloprid-exposed cultures in 34, 48, and 67 genes, respectively.
- Common to all exposed groups were nine genes（four genes up, five genes down) essential for neurodevelopment.
- Chronic neonicotinoid exposure alters the transcriptome of the developing mammalian brain in a similar way to nicotine exposure.



Overviews of gene expression changes in cerebellar cultures exposed to nicotine (NIC), acetamiprid (ACE), and imidacloprid (IMI) for 14days

Acetamiprid accumulates in special site of murine brain (Terayama 2016).

- Mospilan SP (18% acetamiprid + surfactant, dimethyl sulfoxide).
- Mice were fed by water for 3 to 7 days.
  - Normal: water
  - Vehicle: water with dimethyl sulfoxide
  - Acetamiprid: Mospilan (100-fold NOAEL acetamiprid)



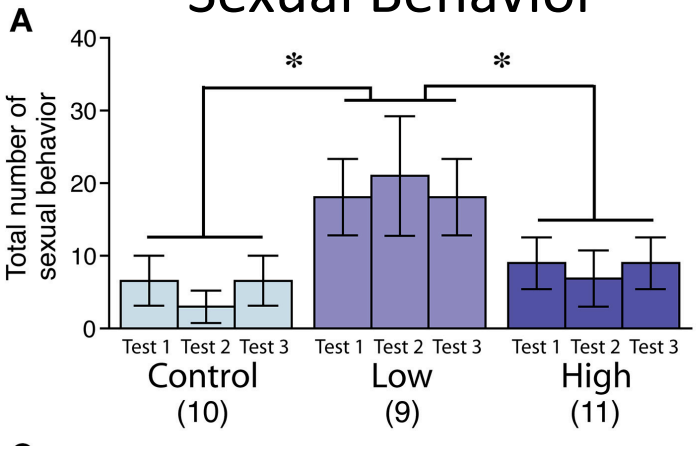
Based on Terayama 2016.

Acetamiprid was also detected from control mouse!

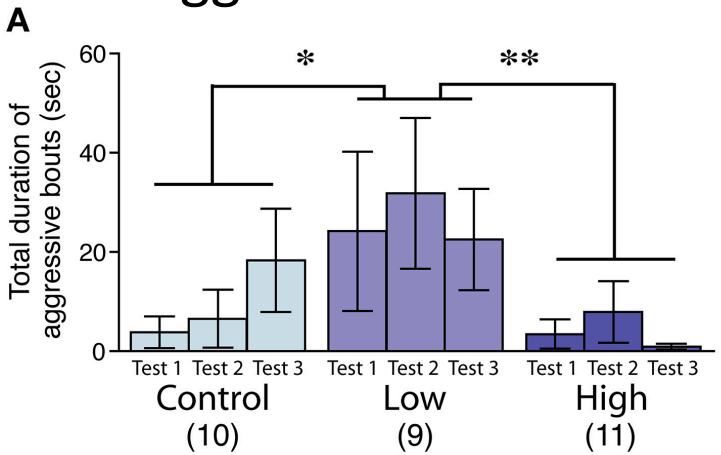
Acetamiprid Induces Abnormalities in Socio-Sexual and Anxiety-Related Behaviors of Male Mice (Sano 2016)

- In utero and lactational Exposure, 0 mg/kg (control group), 1.0 mg/kg (low-dose group), or 10.0 mg/kg (high-dose group)

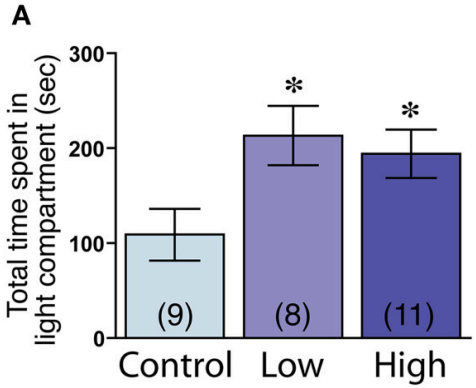
Sexual Behavior



Aggressive behavior



- Anxiety-related behavior, as measured in the light-dark transition



No reductions in the testosterone level, the number of vasopressin-immunoreactive cells, or behavioral flexibility

Neo-nicotinic symptoms have been observed in Gunma, Japan, since 2004 (Taira 2006-2014)

Neo-nicotinic symptoms (NNS)

Subjective Symptoms

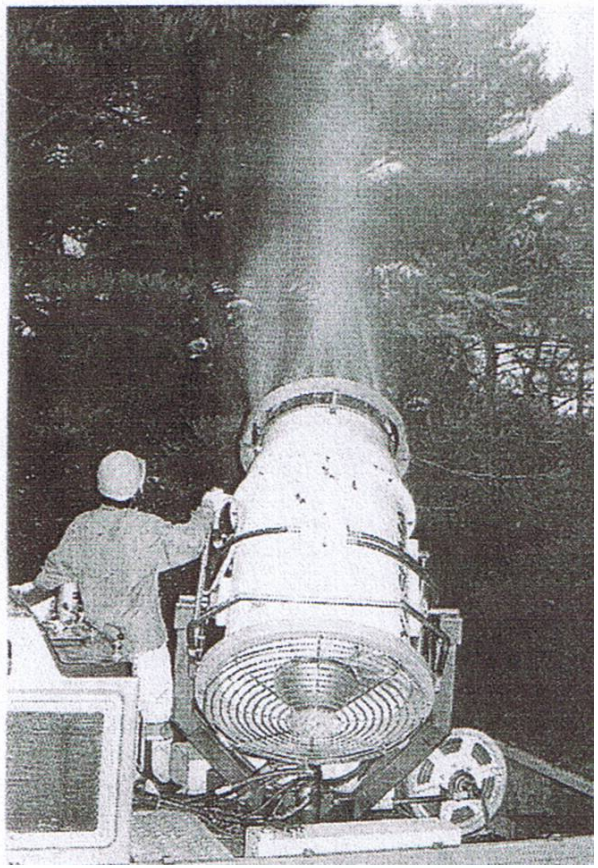
- Headache, General fatigue,
- Stomachache
- Chest pains/palpitation
- Muscle pain/weakness/spasm
- Cough

Objective Symptoms

- Postural tremor
- Recent memory loss
- Fever

First was Acetamiprid Spray in 2004 (Taira 2006)

- 0.02% acetamiprid aqueous solution was sprayed to a height of 40 m or higher above the ground, on mountainsides with air blast spraying equipment, for pine trees as a countermeasure against pine wilt disease.

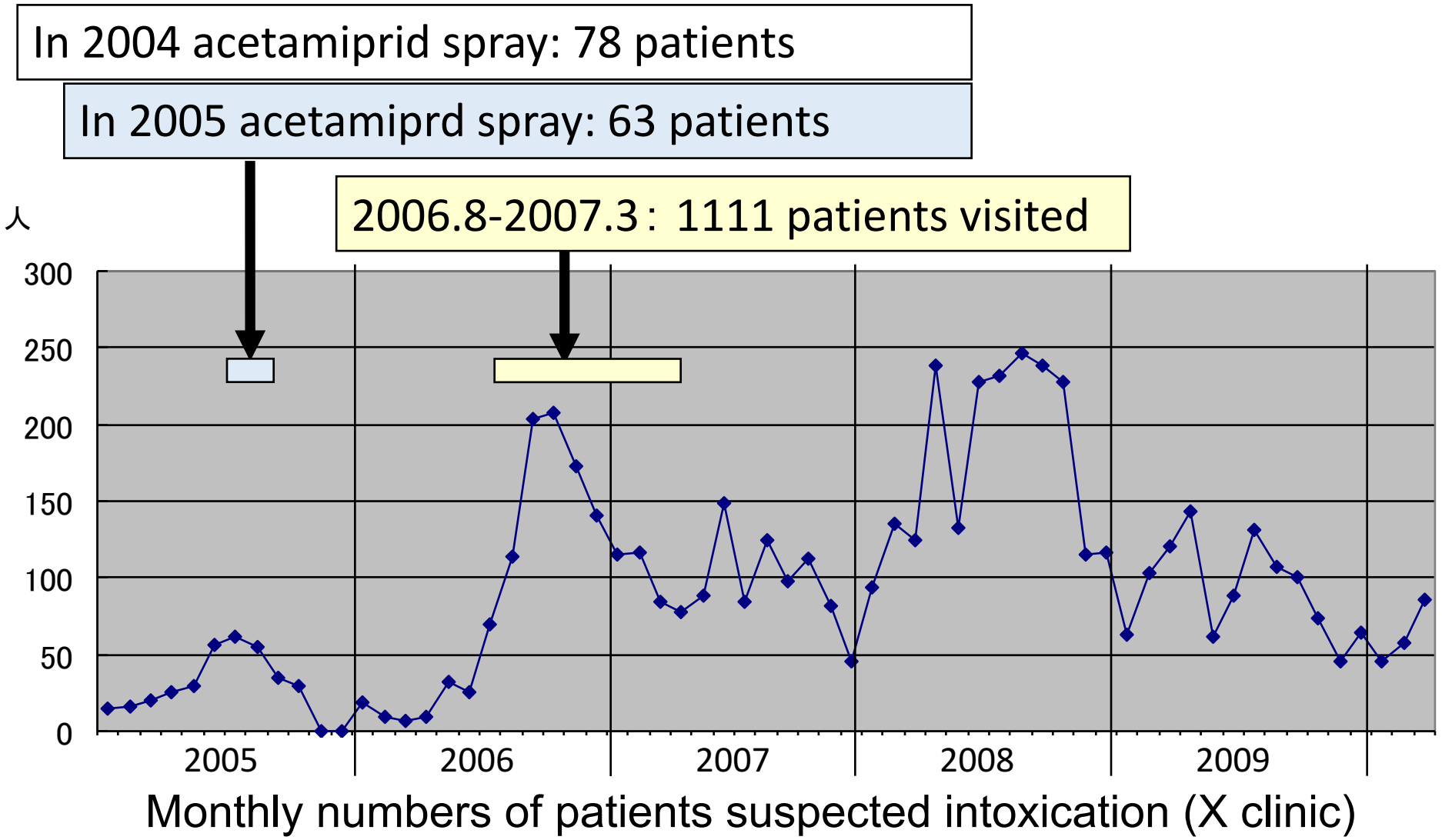


Demographic Data in 2004 and in 2005 (Taira 2014)

	2004	2005
Sprayed Pesticide	Acetamiprid（+OP）	Acetamiprid
Sprayed period	5.26-6.28	5.17-6.24
Acetamiprid per area (μg/m^2)	70	45
Number of patients	78	63
Male/Female	20/58	18/45
Age	2-62	3-78
Under 15 years old	32(50%)	15(26%)
Electrocardiogram findings		
Heart rate abnormality(%)	32 (41%)	18 (29%)

Estimated exposure dose was max. 84.1μg/kg BW, 84% of ARfD (Ichikawa 2008)

After stop spraying in 2006, pandemic of NNS started. All of them became ill after continuous intake of tea beverages and conventional domestic fruits.



We start to analyze patients' urine. Several neonicotinoids and metabolites were identified (Taira et al. 2007-2011).

- Quantified by LC/MS
  - 6-Chloronicotinic acid, maximum 84.8 ng/mL
- Qualified by LC/TOFMS
  - Acetamiprid
  - 5-Hydroxy-imidacloprid
  - 4,5-Dehydro-imidacloprid
  - 4,5-Dihydroxy-imidacloprid
  - *N*-Desmethyl-clothianidin
  - *N*-(2-Chlorothiazole-5-carboxyl)-glycine
- Quantified by LC/MS/MS
  - *N*-Desmethyl-acetetamiprid (DMAP), maximum 3.2 ng/mL

We conducted prospective case control study (Marfo 2015).

Neo-nicotinic symptoms (NNS)

Subjective NNS: headache · general fatigue · stomachache · chest pains/palpitation · muscle pain/weakness/spasm · cough

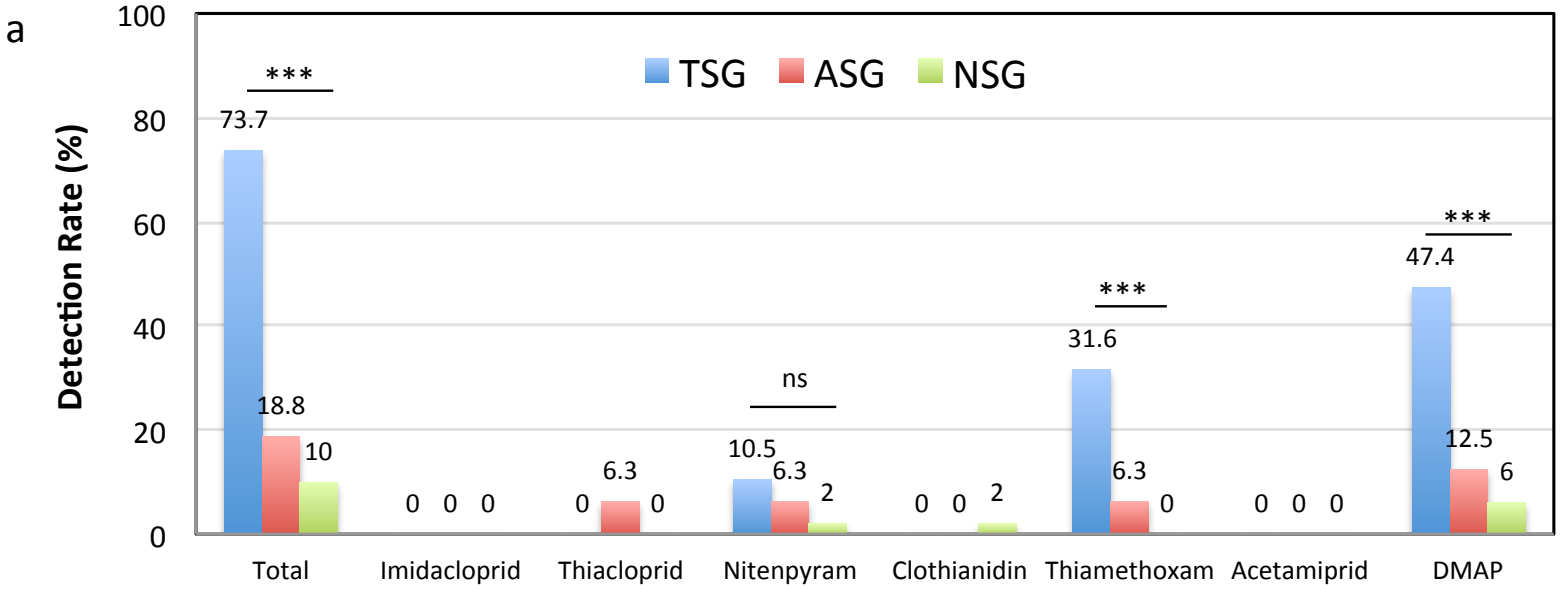
Objective NNS: Postural tremor · Recent memory loss · Fever

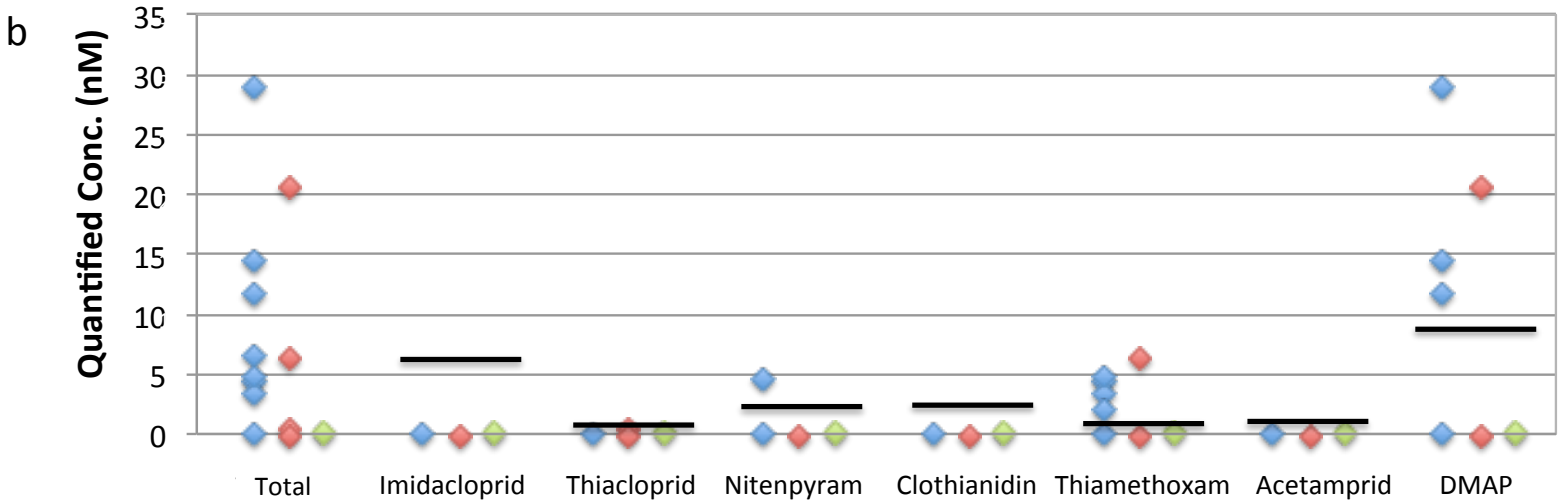
+++	+	-
TSG, n=19	ASG, n=16	NSG, n=50
5 or 6 subjective NNS and Postural tremor(+) and Memory loss (+)	1-4 subjective NNS or Postural tremor (-) or Memory loss (-)	No NNS

Demographic data of each group

Group	TSG	ASG	NSG	P value (TSG vs. NSG)
n	19	16	50	
Sex (M/F)	6/13	6/10	13/37	0.871
Age (y.o.)				
4-10	1	3	4	
10-14	5	3	4	
15-49	8	6	30	
50-64	4	2	6	
65-	1	2	6	
mean ± SD	33.4 ± 21.0	30.9 ± 23.0	39.3 ± 20.1	0.287
min-max	5-69	5-78	4-87	

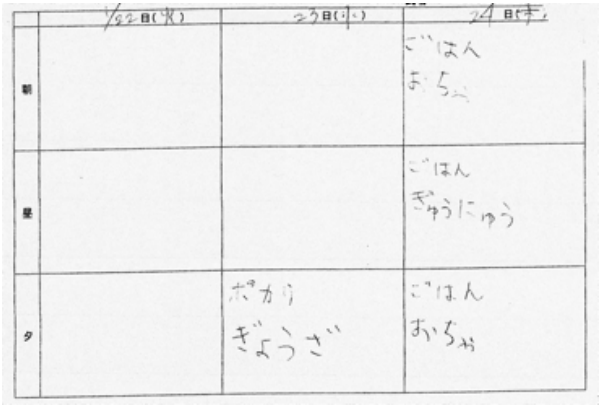
DMAP and Thiamethoxam were more frequently detected in TSG. (prevalence odds ratio=14, 95%C.I. 3.5-57)





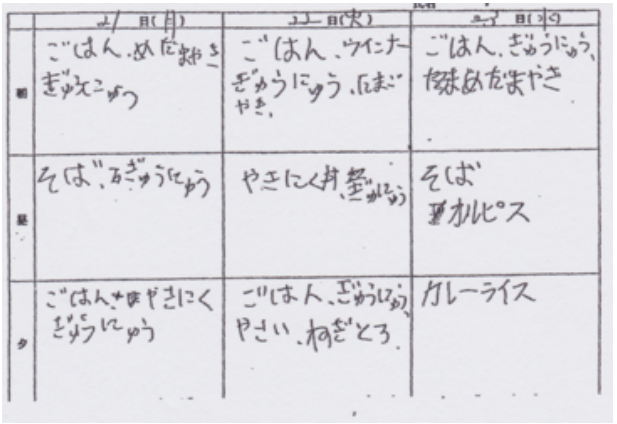
Recent memory loss diagnosed by food diary

Case A: 11 years old female could not recall what she ate for lunch two days ago or before; and DMAP was quantified at 3.6 nmol/mmol Cr in her urine.



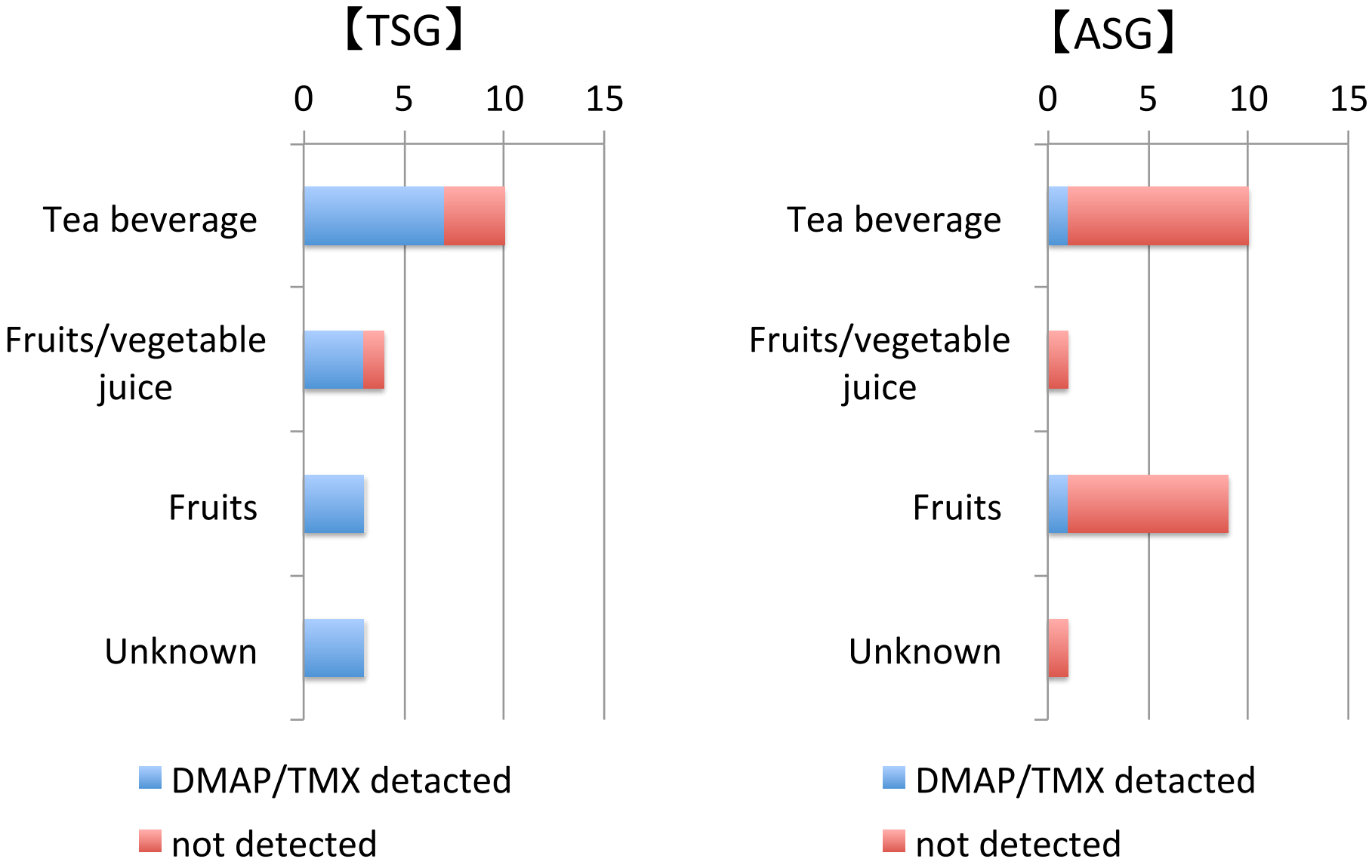
Meal	3 days before	2 days before	1 day before
The first			Rice, tea
The second			Rice, milk
The third		Isotonic water, meat dumpling	Rice, tea

Case B: 11 years old female without neo-nicotinic symptoms



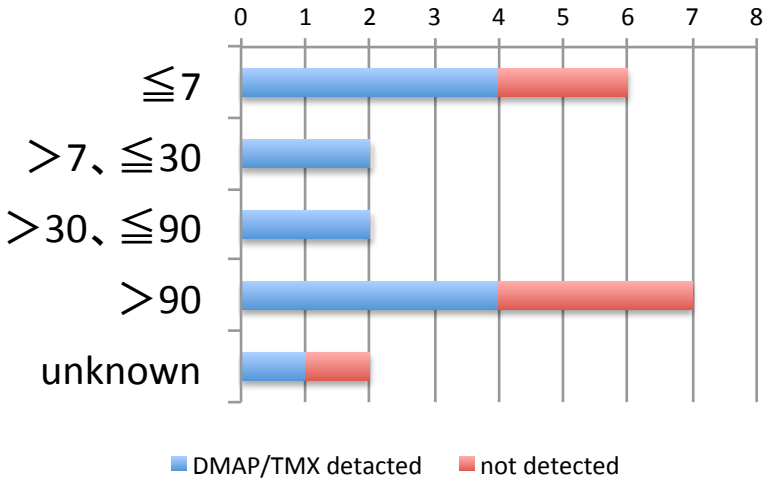
Meal	3 days before	2 days before	1 day before
The first	Rice, grilled egg, milk	Rice, sausage, milk, grilled egg	Rice, milk, grilled egg
The second	Buckwheat noodle, milk	Grilled beef rice, milk	Buckwheat noodle, yogurt drink
The third	Rice, grilled beef, milk	Rice, milk, vegetables, tuna &Welsh onion	Curry and rice

Food intake reported by patients

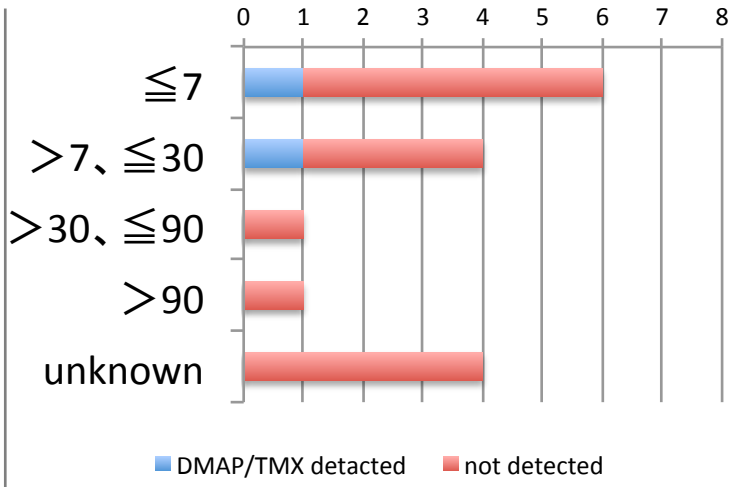


Onset-First visit days (upper) & First visit-remission days (lower)

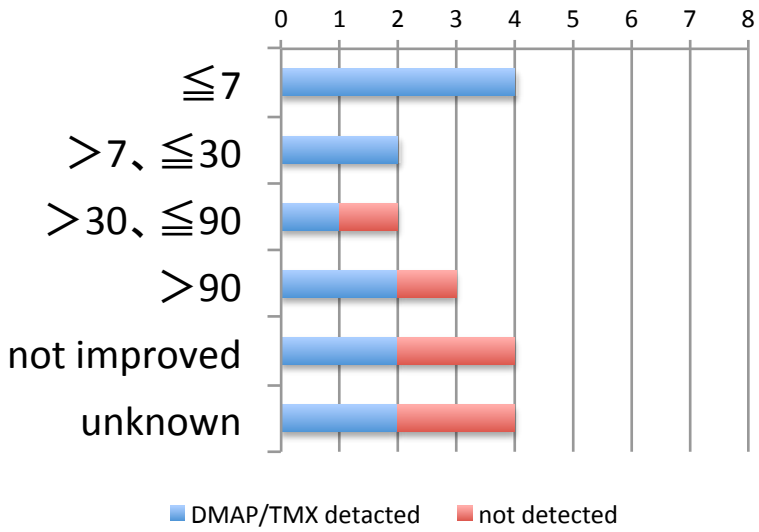
【TSG】

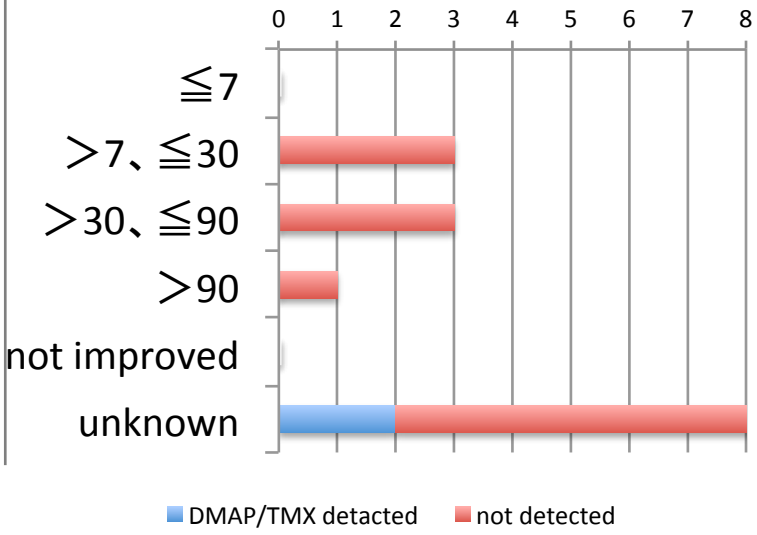


【ASG】



*reported by patients





Toxicokinetics study 1

- **Neonicotinoids are highly soluble to polar solvent.**
  - **High value of acetone/water coefficient may predict accumulation in some part of body.**
- **Imidacloprid has moderate affinity with albumin and hemoglobin (Ding 2015)**

Solubility (g/L)	Water	Octanol	Acetone	K_{aw}
Thiamethoxam	4.1	0.62	48	12
Clothianidin	0.327	1.64	15.2	46
Acetamiprid	2.95	18.5	>200	68
Imidacloprid	0.48	0.78	47	98
Thiacloprid	0.185	3.36	64	346
Nitenpyram	840	192	290	0.35
Dinotefuran	40		58	1.5
Sulfoxaflor	5.7	36.0	217	38
Flupyradifurone	3.2		250	78

US EPA Fact Sheet. 2002;2003;2004, NPIC 2012, Federal Resist 78:18512

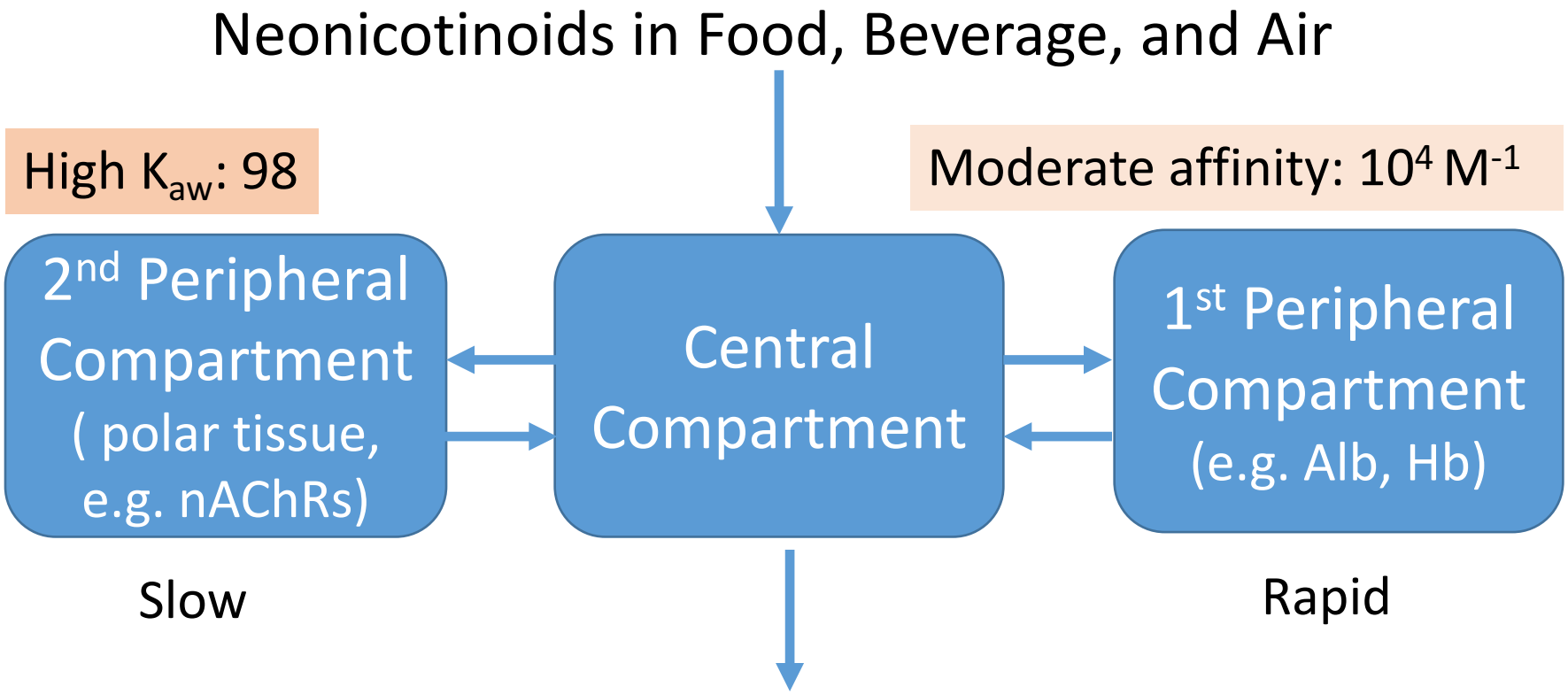
Toxicokinetics study 2

Urinary excretion of imidacloprid and acetamiprid is slow (5μg oral, M/F=5/5, Harada 2016).

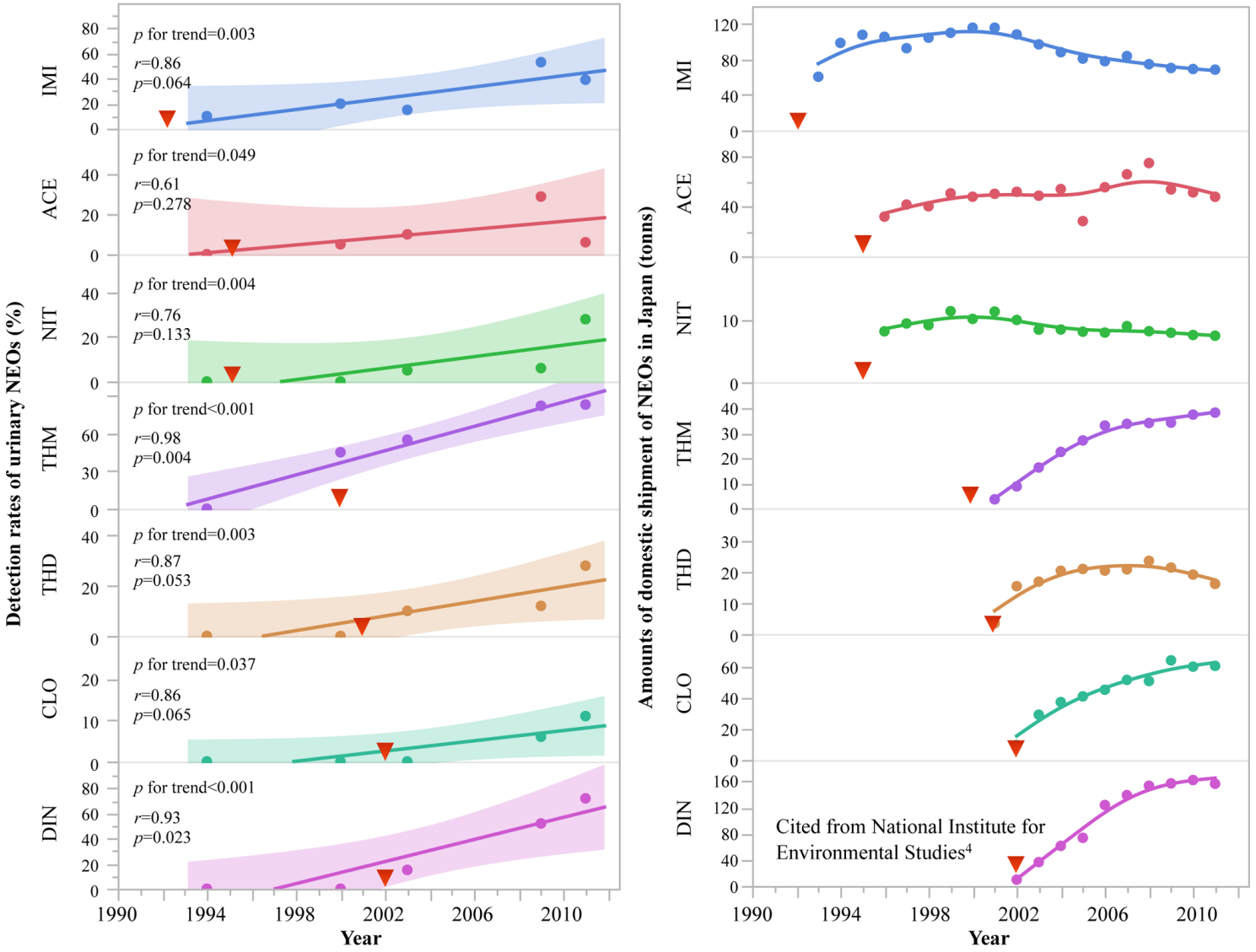
	Design	Bio-availability	r	$T_{1/2}\alpha$ (day)	$T_{1/2}\beta$ (day)
Imidacloprid	adult		0.13	1.45	
	rat blood(M/F)	100%		0.11/0.14	4.91/1.66
Clothianidin	adult		0.60	0.58	
	rat (M/F)	89.2%		0.05/0.06	2.25/0.94
Dinotefuran	adult		0.90	0.17	
	rat (M/F)	98.5-98.9%		0.15/0.33	
dm-Acetamiprid	adult		0.59	0.23	1.65
	rat (M/F)	100%		no data	no data

r : Area under the curve (AUC), proportion excreted in urine

Plausible Neonicotinoid Toxicokinetics

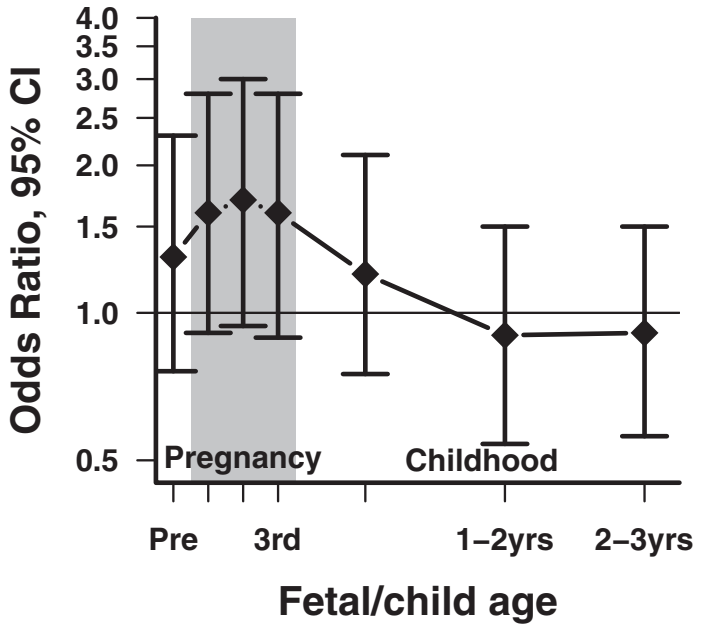


Detection rates of urinary neonicotinoids are increasing in Japan (Ueyama 2015). That of 3 y.o. is 58% (Osaka2016)



Autism and imidacloprid, a common flea and tick treatment for pets (Keil 2014)

- CHARGE (Childhood Autism Risks from Genetics and Environment) case-control study in CA, completed before 2011.
- **The association between imidacloprid exposure and ASD warrants further investigation.**
- **This work highlights the need for validation studies regarding prenatal exposures in ASD.**



Adjusted odds ratios and 95% confidence intervals comparing imidacloprid exposure of children with autism spectrum disorder with typically developing controls from the CHARGE data.

Other studies in CA, San Joaquin Valley

- Children who was born from 1997 to 2006
- Examined the mothers exposed to neonicotinoids within a 500 m radius of their address during a 3-month periconceptional window, and not exposed.

- Congenital heart defects (Carmichael et al. 2014)
  - 101 Tetralogy of Fallot and 785 non-malformed controls
  - Adjusted Odds Ratio (95% CI) was 2.4(1.1–5.1)
- Anencephaly (Yang et al.2014)
  - 73 anencephaly and 785 non-malformed controls
  - Adjusted Odds Ratio (95% CI) was 2.5 (0.9–7.1)

Regulation

Process of Pesticide risk management

1. Collect evidence
   - case study, case report,
   - animal study, laboratory data, structure activity correlation
2. Find dose response relationship
   - Acute Reference Dose (ARfD, single dose)
   - Acceptable Dose of Intake (ADI, speed)
3. Evaluate the level of exposure
4. Assess the risk

WHO classified pesticide by acute toxicity in 2009. However, appropriate ADI setting may also protect children from the hazard of pesticides.

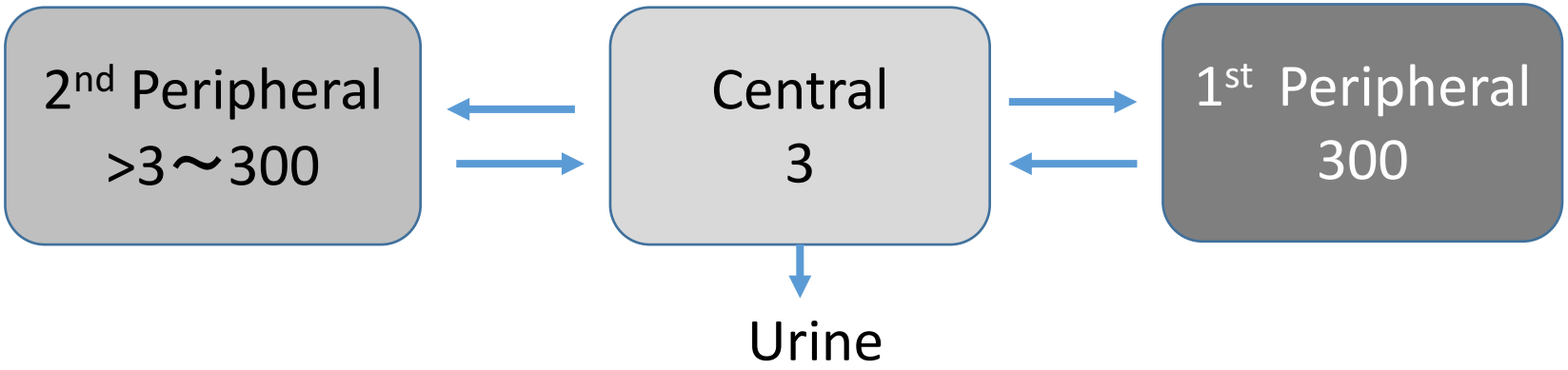
Lowest-observed-adverse-effect level (LOAEL) of neonicotinoids in human study

Neonicotinoid	Exposure	Dose (µg/kg)	Matrix	Level (µg/L)	Current ADI (µg/kg)
Imidacloprid	Acute	8300	Blood	3	57
dm-Acetamiprid	Chronic		Urine	< 0.6	71
Thiamethoxam	Chronic		Urine	< 0.3	18

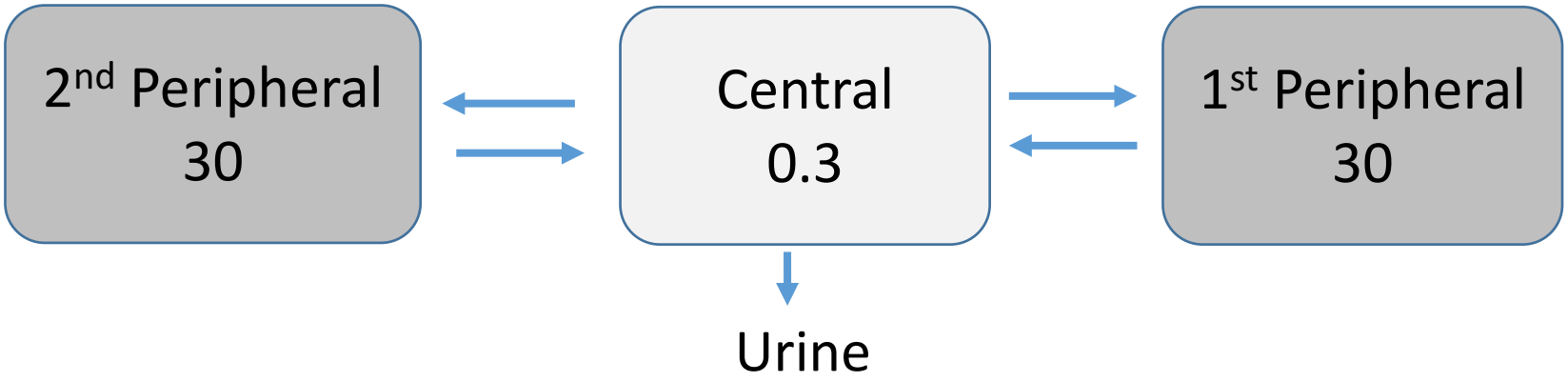
- **Imidacloprid acute intoxication (Tamura 2002)**
  - **95 y.o. male, entubated, gastric lavage in ER**
  - **2% formula 25 mL, oral (8300 µg/kg)**
  - **LOAEL = 8300 µg/kg**
  - **ARfD = 8300/10/10=83 µg/kg**
  - **ADI = ARfD/10 = 8.3 µg/kg = <15% of current ADI**

Why urinary excretion is low in chronic exposure?

Acute exposure



Chronic exposure



What we can do to reduce environmental neonicotinoids exposure?

- Reduce ADI?
- Share ADI with all neonicotinoids?
- Ban all?
- Restrict the application?
- Stop seed treatment by neonicotinoids?
- Use alternatives?
- Go organic for sustainable agriculture?

- **It is the time we stop the challenge test on child brain.**

Conclusion

- Recent animal studies and in vitro studies suggest neonicotinoids may cause neurodevelopmental disorders.
- We have several human evidence about the health effect of neonicotinoid by environmental exposure.
- Acceptable Dose of Intake of neonicotinoids needs to be revised to protect children from neurodevelopment disorders.

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Regulation

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