

# Air monitoring in PACA Region genotoxicity of pesticide 'cocktails'

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## Context

Chronic exposure to pesticides is suspected to have multiple impacts on human health (hematopoietic cancers, neurodegenerative and fertility disorders...). These pathologies are often considered to result, at least in part, from long-term exposures to compounds whose effects, in terms of physiological disorders, are particularly delayed.

Although genotoxic effects of each pesticide have been investigated before market authorization, the genotoxic properties of pesticide mixtures are poorly documented. Indeed, human population are frequently exposed to a combination of chemicals including pesticides, and the contribution of these mixtures to an increase risk of cancer, neurodegenerative and / or fertility disorders should be addressed.

## Mixtures of pesticides

MIX 1	MIX 2	MIX 3	MIX 4	MIX 5	MIX 6	MIX 7
Lindane	Lindane	Lindane	Lindane	Lindane	Lindane	Lindane
Chlorpyrifos	Chlorpyrifos	Chlorpyrifos	Chlorpyrifos	Chlorpyrifos	Chlorpyrifos	Chlorpyrifos
Folpet	Folpet		Folpet	Folpet	Folpet	Folpet
Pendimethalin	Pendimethalin		Pendimethalin	Pendimethalin	Pendimethalin	Pendimethalin
Tebuconazole	Tebuconazole	Tebuconazole	Tebuconazole	Tebuconazole	Tebuconazole	Tebuconazole
	Piperonyl butoxide	Piperonyl butoxide		Piperonyl butoxide		Piperonyl butoxide
					Difenoconazole	Difenoconazole
			Diflufenican	Diflufenican	Diflufenican	Diflufenican

Mixtures most likely to be found on the 5 sampling sites

## Pesticide monitoring

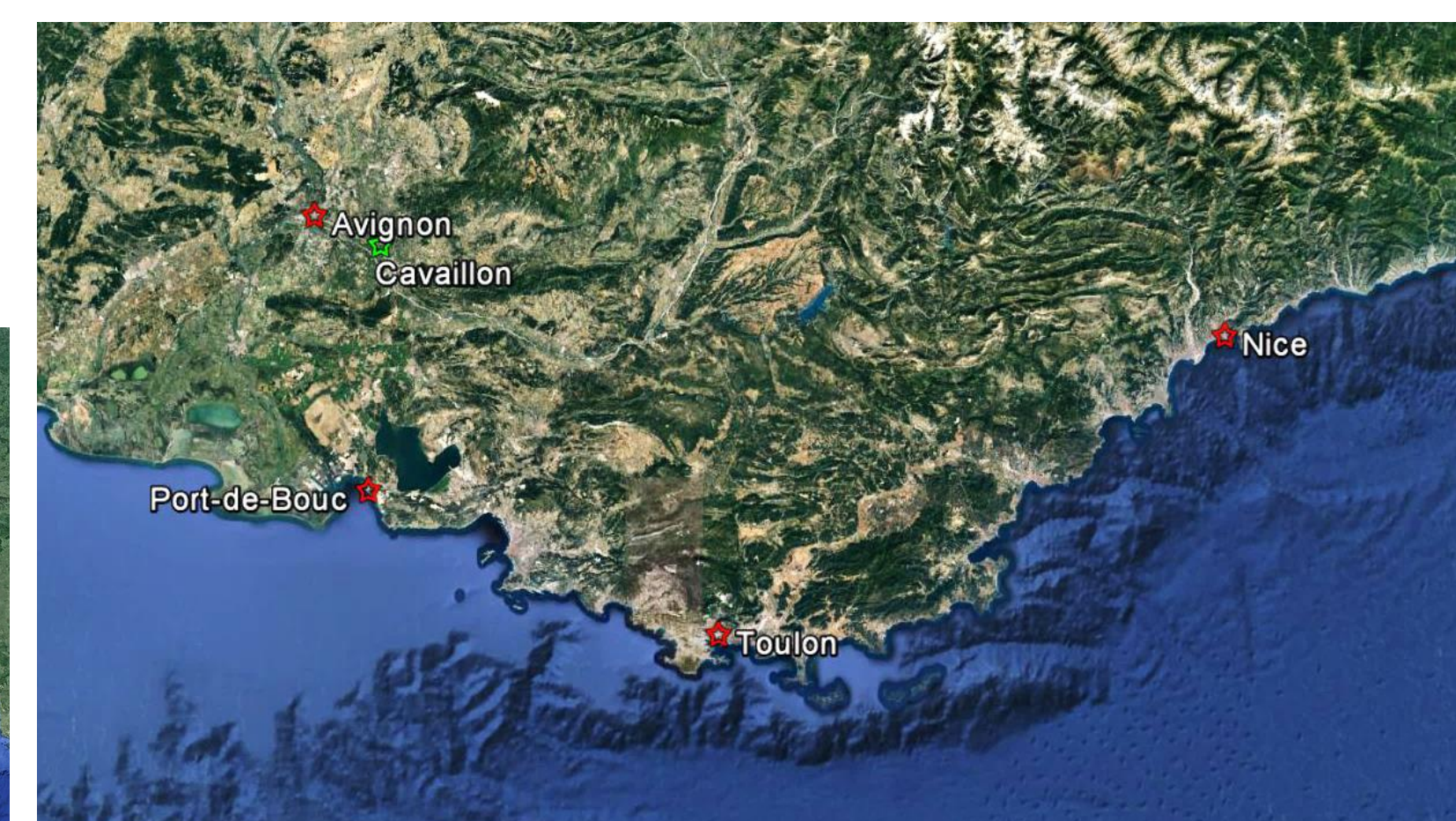
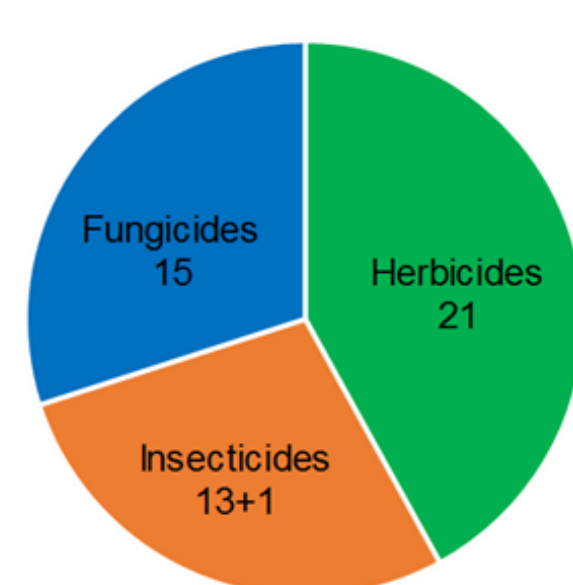
This work characterizes the genotoxic potential of various mixtures of pesticides defined from atmospheric concentrations of pesticides monitored in 2014 in the French Provence-Alpes-Côte d'Azur Region.

Sampling: 50 pesticides; 120 samples

high-volume sampler (10 m<sup>3</sup> h<sup>-1</sup> for 48 h)

→ gas-phase (polyurethane foams - PUF)

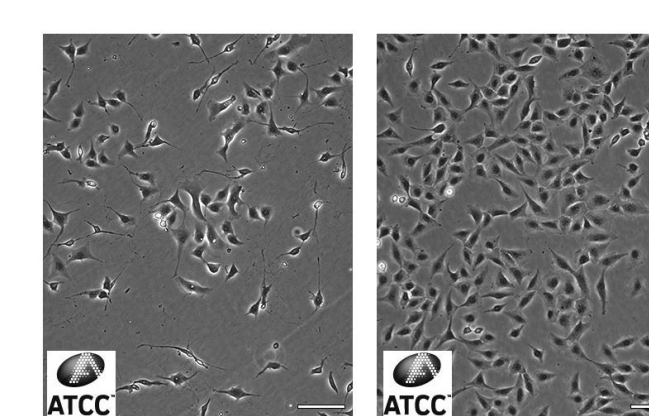
→ particle-phase (Total Suspended Particulate ; quartz fibre filter)



## Cell culture

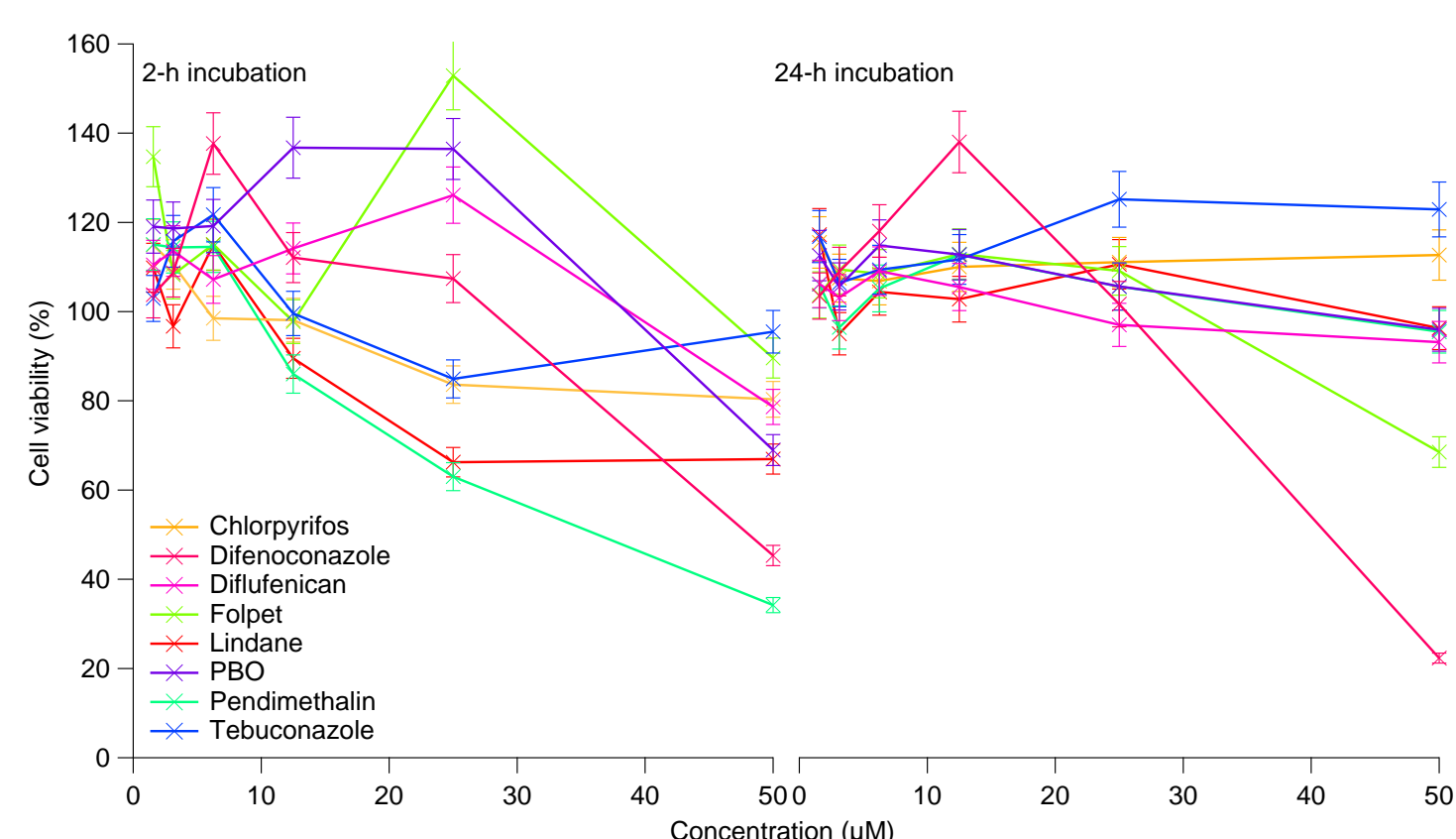
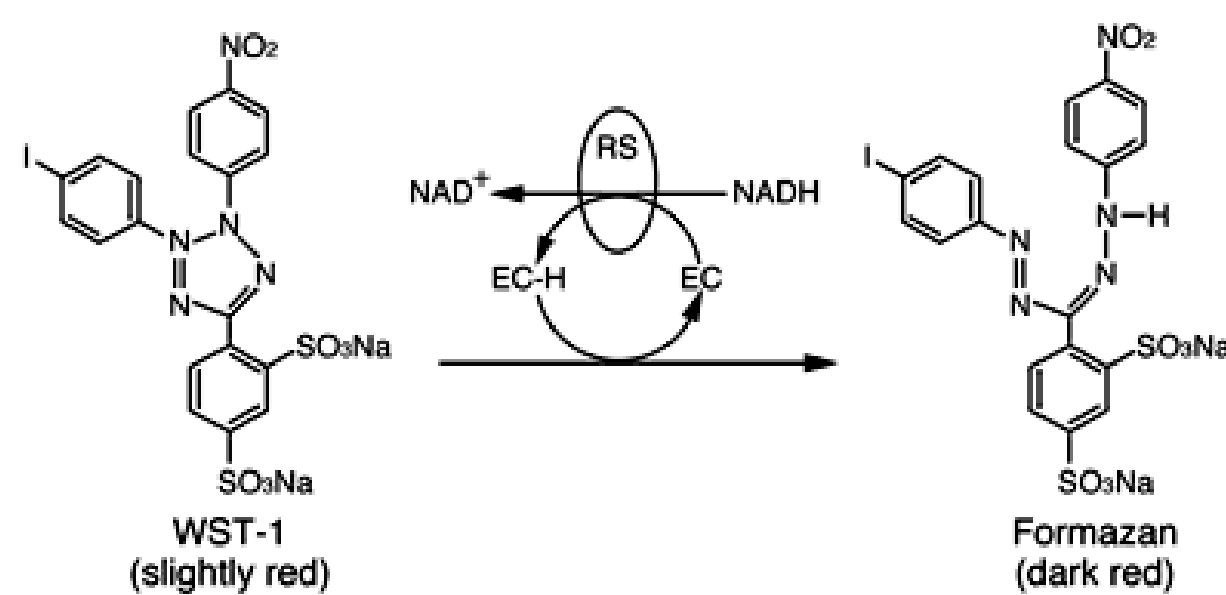
Cells: Human bronchial epithelial BEAS-2B

Culture medium: LHC-9 (37°C, 5% CO<sub>2</sub>)

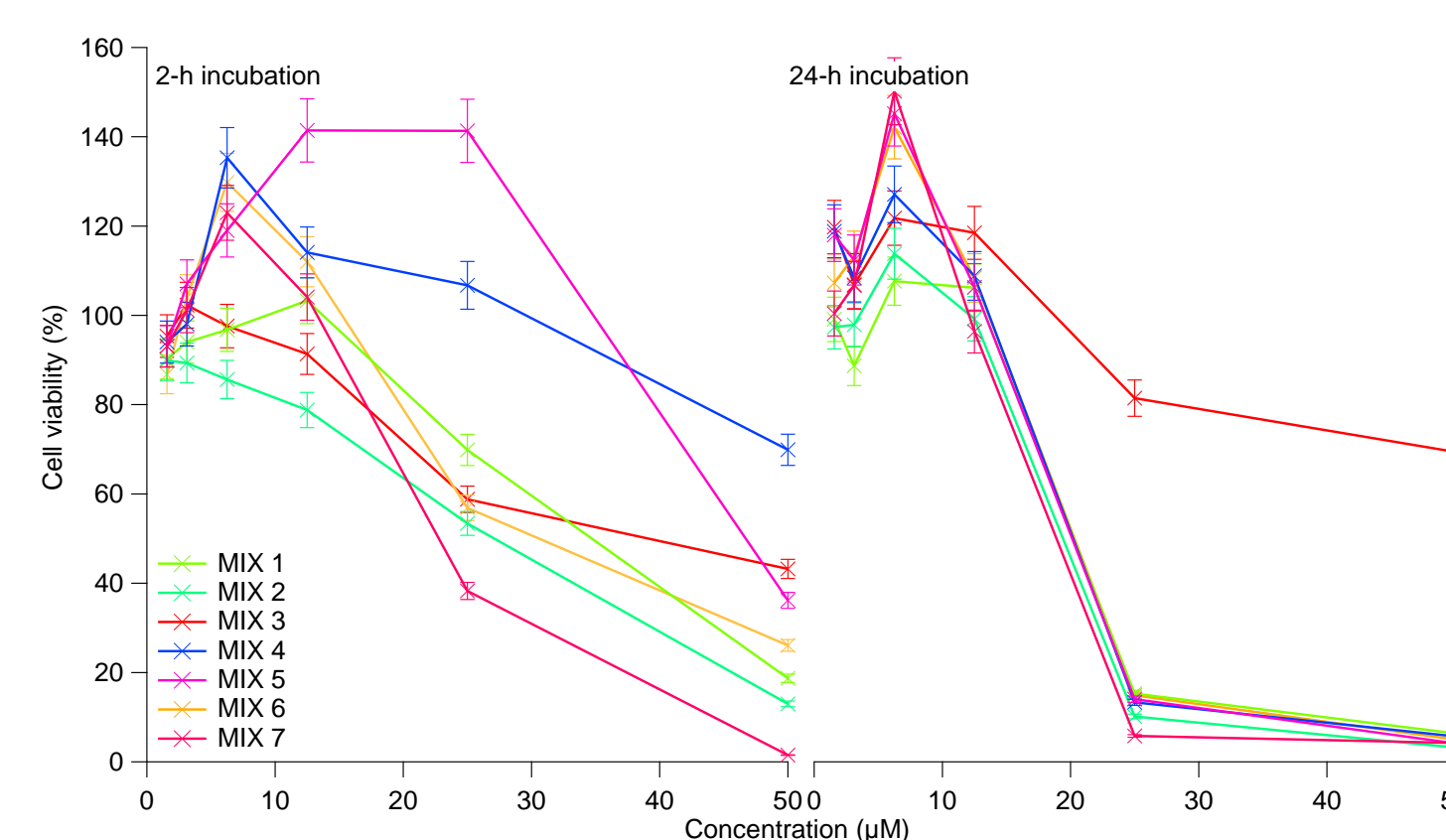


## Cytotoxicity test

1. WST-1 test: to define the concentration ranges to be tested in genotoxicity



Difenconazole and pendimethalin: viability < 50% (50 µM)



Increase of the cytotoxicity for the mixtures

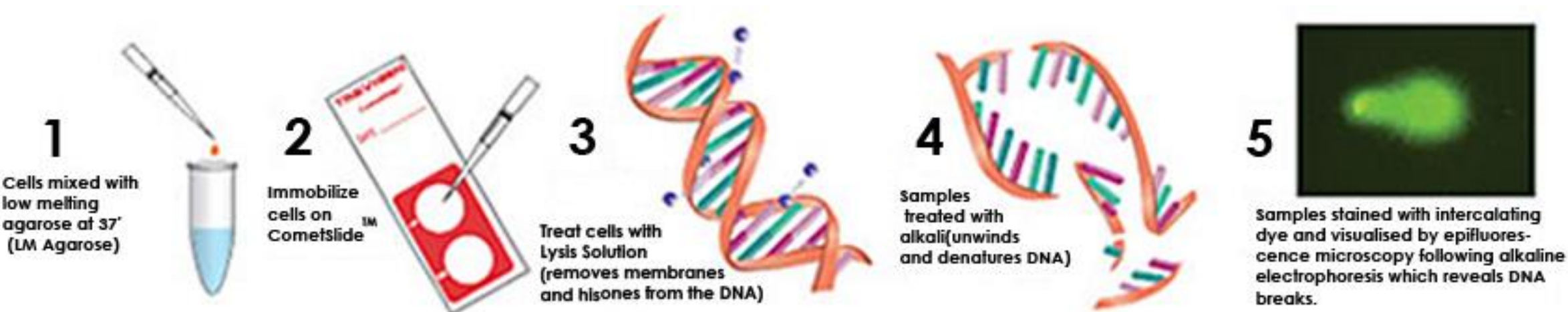
Individual pesticides: no to moderate cytotoxic effects after 2-h and 24-h incubation

Pesticides cocktails: moderate to severe cytotoxic effects after 2-h and 24-h incubation

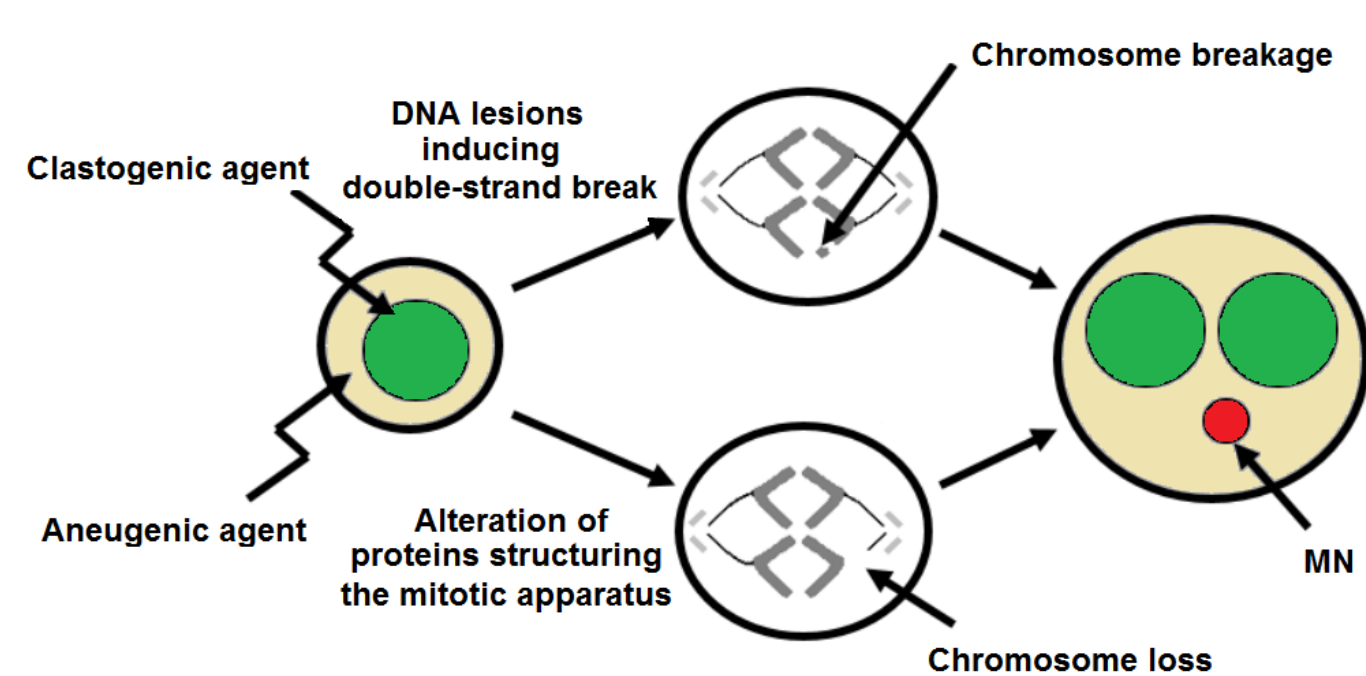
Concentration range to be used in the micronucleus assay: 1.5 to 12.5 µM

## Genotoxicity tests (in vitro)

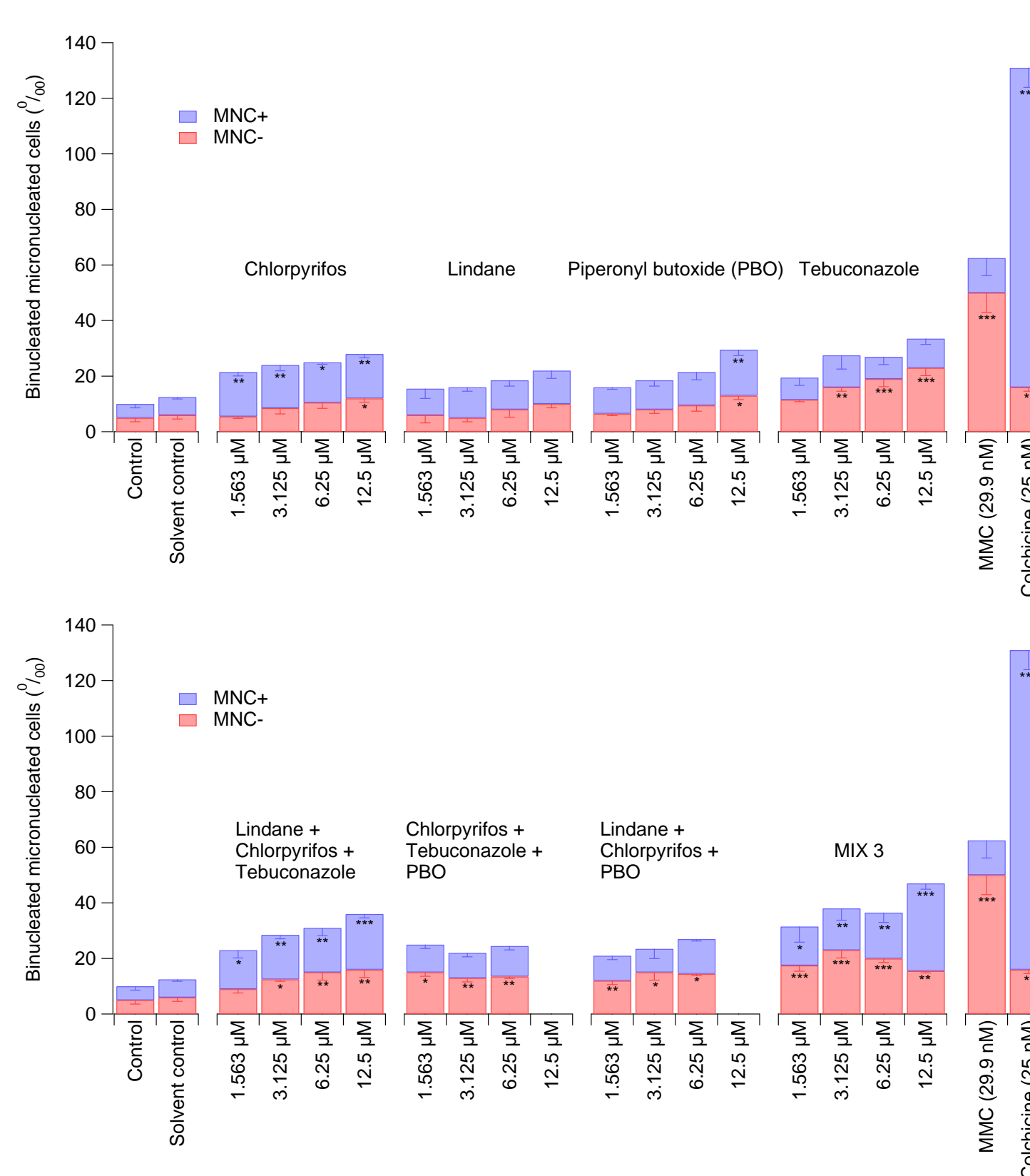
2. Comet assay: detection of DNA damage



3. Micronucleus test: indication of chromosome damage



Look at MIX 3



Chromosome breakage induction were noted following exposure to Chlorpyrifos at concentrations ≥ 1.5 µM and to PBO at 12.5 µM only

Chromosome losses induction were noted following exposure to Chlorpyrifos and PBO at 12.5 µM only and to Tebuconazole at concentrations ≥ 3.1 µM

In mixtures, both chromosome breakage and losses were induced in a dose-dependent manner for (Lindane + Chlorpyrifos + Tebuconazole) and for MIX3

### Summary of results

Mixtures of pesticides	Comet assay		Micronucleus test	
	2h incubation	24h incubation	MNC-	MNC+
MIX 1	++	-	+/-	+
MIX 2	+	+	+	+/-
MIX 3	-	+	++	+
MIX 4	++	+/-	-	-
MIX 5	+++	++	-	-
MIX 6	+++	++	+/-	-
MIX 7	+++	+++	+	-

Most of the MIX tested induced a positive response in the comet assay, that is primary DNA lesions.

Among the 7 MIX tested, 3 of them clearly induced chromosome losses (MNC+) whereas 2 induced chromosome breakage

## Conclusion and outlook

Some pesticide mixtures lead to:

- Primary DNA lesions (Comet Assay) and chromosome damage (Micronuclei),
- Chromosome damage consisted in both losses of whole chromosomes or chromosome breaks.

The results could be used to define pesticides that could be used at the same place in the same period and those that could be carefully used one after the other.

Fundings: PACA Region – Air PACA